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(54) Title: OLIGOMERIC COMPOUNDS AND COMPOSITIONS FOR USE IN MODULATION OF SMALL NON-CODING RNAS

(57) Abstract: Compounds, compositions and methods are provided for modulating the expression and function of small non-coding RNAs. The compositions comprise oligomeric compounds, targeted to small non-coding RNAs. Methods of using these compounds for modulation of small non-coding RNAs as well as downstream targets of these RNAs and for diagnosis and treatment of disease associated with small non-coding RNAs are also provided.

WO 2005/013901 A2

OLIGOMERIC COMPOUNDS AND COMPOSITIONS FOR USE IN MODULATION OF SMALL NON-CODING RNAS

Field of the Invention

The present invention provides compositions and methods for modulation of small non-coding RNAs. In particular, this invention relates to compounds, particularly oligomeric compounds, which, in some embodiments, hybridize with or sterically interfere with nucleic acid molecules comprising or encoding small non-coding RNA targets. Such compounds are shown herein to modulate the levels of small non-coding RNAs. The oligomeric compounds of the invention may include one or more modifications thereon resulting in differences in physical or chemical properties compared to unmodified nucleic acids. These modified oligomeric compounds are used as single compounds or in compositions to modulate or mimic the targeted nucleic acid comprising or encoding the small non-coding RNA. In some embodiments of the invention, modifications include chemical modifications that improve activity of the oligomeric compound. In some embodiments, the modifications include moieties that modify or enhance the pharmacokinetic or pharmacodynamic properties, stability or nuclease resistance of the oligomeric compound. In some embodiments, the modifications render the oligomeric compounds capable of sterically interfering with the natural processing of the nucleic acids comprising or encoding the small non-coding RNA targets.

Background of the Invention

RNA genes were once considered relics of a primordial "RNA world" that was largely replaced by more efficient proteins. More recently, however, it has become clear that non-coding RNA genes produce functional RNA molecules with important roles in regulation of gene expression, developmental timing, viral surveillance, and immunity. Not only the classic transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), but also small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), small interfering RNAs (siRNAs), tiny non-coding RNAs (tncRNAs), repeat-associated small interfering RNAs (rasiRNAs) and microRNAs (miRNAs) are now believed to act in diverse cellular processes such as chromosome maintenance, gene imprinting, pre-mRNA splicing, guiding RNA modifications, transcriptional regulation, and the control of mRNA translation (Eddy, Nat. Rev. Genet., 2001, 2, 919-929; Kawasaki and Taira, Nature, 2003, 423, 838-842; Aravin, et al., Dev. Cell, 2003, 5, 337-350). RNA-mediated processes are now also believed to direct heterochromatin formation, genome rearrangements,

and DNA elimination (Cerutti, Trends Genet., 2003, 19, 39-46; Couzin, Science, 2002, 298, 2296-2297).

The recently described phenomenon known as RNA interference (RNAi) is involves the processing of double stranded RNA into siRNAs by an RNase III-like dsRNA-specific enzyme known as Dicer (also known as helicase-moi) which are then incorporated into a ribonucleoprotein complex, the RNA-induced silencing complex (RISC). RISC is believed to use the siRNA molecules as a guide to identify complementary RNAs, and an endoribonuclease (to date unidentified) cleaves these target RNAs, resulting in their degradation (Cerutti, Trends Genet., 2003, 19, 39-46; Grishok et al., Cell, 2001, 106, 23-34). In addition to the siRNAs, a large class of small non-coding RNAs known as microRNAs (miRNAs, originally termed stRNA for "short temporal RNAs") is believed to play a role in regulation of gene expression employing some of the same players involved in the RNAi pathway (Novina and Sharp, Nature, 2004, 430, 161-164).

Like siRNAs, miRNAs are believed to be processed endogenously by the Dicer enzyme, and are approximately the same length, and possess the characteristic 5'-phosphate and 3'-hydroxyl termini. The miRNAs are also incorporated into a ribonucleoprotein complex, the miRNP, which is similar, and may be identical to the RISC (Bartel and Bartel, Plant Physiol., 2003, 132, 709-717). More than 200 different miRNAs have been identified in plants and animals (Ambros et al., Curr. Biol., 2003, 13, 807-818).

In spite of their biochemical and mechanistic similarities, there are also some differences between siRNAs and miRNAs, based on unique aspects of their biogenesis. siRNAs are generated from the cleavage of long exogenous or possibly endogenous dsRNA molecules, such as very long hairpins or bimolecular duplexed dsRNA, and numerous siRNAs accumulate from both strands of dsRNA precursors. In contrast, mature miRNAs appear to originate from long endogenous primary miRNA transcripts (also known as pri-miRNAs, pri-mirs or pri-pre-miRNAs) that are often hundreds of nucleotides in length (Lee, et al., EMBO J., 2002, 21(17), 4663-4670).

The current model of miRNA processing involves primary miRNA transcripts being processed by a nuclear enzyme in the RNase III family known as Drosha, into approximately 70 nucleotide-long pre-miRNAs (also known as stem-loop structures, hairpins, pre-mirs or foldback miRNA precursors) which are subsequently processed by the Dicer RNase into mature miRNAs, approximately 21-25 nucleotides in length. It is believed that, in processing the pri-miRNA into the pre-miRNA, the Drosha enzyme cuts the pri-miRNA at the base of the mature miRNA, leaving a 2-nt 3' overhang (Ambros et al., RNA, 2003, 9, 277-279; Bartel and Bartel, Plant

Physiol., 2003, 132, 709-717; Shi, Trends Genet., 2003, 19, 9-12; Lee, et al., EMBO J., 2002, 21(17), 4663-4670; Lee, et al., Nature, 2003, 425, 415-419) . The 3' two-nucleotide overhang structure, a signature of RNaseIII cleavage, has been identified as a critical specificity determinant in targeting and maintaining small RNAs in the RNA interference pathway (Murchison, et al., Curr. Opin. Cell Biol., 2004, 16, 223-9). Both the primary RNA transcripts (pri-miRNAs) and foldback miRNA precursors (pre-miRNAs) are believed to be single-stranded RNA molecules with at least partial double-stranded character, often containing smaller, local internal hairpin structures. Primary miRNA transcripts may be processed such that one single-stranded mature miRNA molecule is generated from one arm of the hairpin-like structure of the pri-miRNA. Alternatively, a polycistronic pri-miRNA may contain multiple pre-miRNAs, each processed into a different, single-stranded mature miRNA.

Naturally occurring miRNAs are characterized by imperfect complementarity to their target sequences. Artificially modified miRNAs with sequences completely complementary to their target RNAs have been designed and found to function as double stranded siRNAs that inhibit gene expression by reducing RNA transcript levels. Synthetic hairpin RNAs that mimic siRNAs and miRNA precursor molecules were demonstrated to target genes for silencing by degradation and not translational repression (McManus et al., RNA, 2002, 8, 842-850).

Tiny non-coding RNA (tncRNA), one class of small non-coding RNAs (Ambros et al., Curr. Biol., 2003, 13, 807-818) produce transcripts similar in length (20-21 nucleotides) to miRNAs, and are also thought to be developmentally regulated but, unlike miRNAs, tncRNAs are reportedly not processed from short hairpin precursors and are not phylogenetically conserved. Although none of these tncRNAs are reported to originate from miRNA hairpin precursors, some are predicted to form potential foldback structures reminiscent of pre-miRNAs; these putative tncRNA precursor structures deviate significantly from those of pre-miRNAs in key characteristics, i.e., they exhibit excessive numbers of bulged nucleotides in the stem or have fewer than 16 base pairs involving the small RNA (Ambros et al., Curr. Biol., 2003, 13, 807-818).

Recently, another class of small non-coding RNAs, the repeat-associated small interfering RNAs (rasiRNAs) has been isolated from *Drosophila melanogaster*. The rasiRNAs are associated with repeated sequences, transposable elements, satellite and microsatellite DNA, and Suppressor of Stellate repeats, suggesting that small RNAs may participate in defining chromatin structure (Aravin, et al., Dev. Cell, 2003, 5, 337-350).

A total of 201 different expressed RNA sequences potentially encoding novel small non-messenger species (smnRNAs) has been identified from mouse brain cDNA libraries. Based

on sequence and structural motifs, several of these have been assigned to the snoRNA class of nucleolar localized molecules known to act as guide RNAs for rRNA modification, whereas others are predicted to direct modification within the U2, U4, or U6 small nuclear RNAs (snRNAs). Some of these newly identified smnRNAs remained unclassified and have no identified RNA targets. It was suggested that some of these RNA species may have novel functions previously unknown for snoRNAs, namely the regulation of gene expression by binding to and/or modifying mRNAs or their precursors via their antisense elements (Huttenhofer et al., Embo J., 2001, 20, 2943-2953).

To date, the binding and regulatory sites within nucleic acid targets of the small non-coding RNAs are largely unknown, although a few putative motifs have been suggested to exist in the 3'UTR of certain genes (Lai and Posakony, Development, 1997, 124, 4847-4856; Lai, et al., Development, 2000, 127, 291-306; Lai, Nat Genet. 2002, 30(4), 363-364).

One miRNA is also believed to act as a cell death regulator, implicating it in mechanisms of human disease such as cancer. Recently, the *Drosophila* mir-14 miRNA was identified as a suppressor of apoptotic cell death and is required for normal fat metabolism. (Xu et al., Curr. Biol., 2003, 13, 790-795).

Downregulation or deletion of other miRNAs has been associated with B-cell chronic lymphocytic leukemia (B-CLL) (Calin et al., Proc. Natl. Acad. Sci. USA, 2002, 99, 15524-15529), and human homologues of the murine mir-143 and mir-145 mature miRNAs were recently reported to be expressed and processed at reduced steady-state levels at the adenomatous and cancerous stages of colorectal neoplasia (Michael, et al., Mol. Cancer Res., 2003, 1, 882-891).

Expression of the human mir-30 miRNA specifically blocked the translation in human cells of an mRNA containing artificial mir-30 target sites. In these studies, putative miRNAs were excised from transcripts encompassing artificial miRNA precursors and could inhibit the expression of mRNAs containing a complementary target site. These data indicate that novel miRNAs can be readily produced *in vivo* and can be designed to specifically inactivate the expression of selected target genes in human cells (Zeng et al., Mol. Cell, 2002, 9, 1327-1333).

Disclosed and claimed in PCT Publication WO 03/029459 are miRNAs from several species,or a precursor thereof; a nucleotide sequence which is the complement of said nucleotide sequence which has an identity of at least 80% to said sequence; and a nucleotide sequence which hybridizes under stringent conditions to said sequence. Also claimed is a pharmaceutical composition containing as an active agent at least one of said nucleic acid and optionally a pharmaceutically acceptable carrier, and a method of identifying microRNA molecules or

precursor molecules thereof comprising ligating 5'-and 3'-adapter molecules to the ends of a size-fractionated RNA population, reverse transcribing said adapter containing RNA population and characterizing the reverse transcription products (Tuschl et al., Genes Dev., 1999, 13, 3191-3197).

Small non-coding RNA-mediated regulation of gene expression is an attractive approach to the treatment of diseases as well as infection by pathogens such as bacteria, viruses and prions and other disorders associated with RNA expression or processing.

Consequently, there remains a long-felt need for agents that regulate gene expression via the mechanisms mediated by small non-coding RNAs. Identification of modified miRNAs or miRNA mimics that can increase or decrease gene expression or activity is therefore desirable.

The present invention therefore provides oligomeric compounds and methods useful for modulating gene levels, expression, function or pathways, including those relying on mechanisms of action such as RNA interference and dsRNA enzymes, as well as antisense and non-antisense mechanisms. One having skill in the art, once armed with this disclosure will be able, without undue experimentation, to identify compounds, compositions and methods for these uses.

Summary of the Invention

The present invention provides oligomeric compounds, especially nucleic acid and nucleic acid-like oligomers, which are targeted to or mimic nucleic acids comprising or encoding small non-coding RNAs, and which act to modulate the levels of small non-coding RNAs, or interfere with their function.

The present invention also provides oligomeric compounds comprising a first strand and a second strand wherein at least one strand contains a modification and wherein a portion of one of the oligomeric compound strands is capable of hybridizing to a small non-coding RNA target nucleic acid.

The present invention also provides oligomeric compounds comprising a first region and a second region and optionally a third region wherein at least one region contains a modification and wherein a portion of the oligomeric compound is capable of hybridizing to a small non-coding RNA target nucleic acid.

The present invention also provides oligomeric compounds, especially nucleic acid and nucleic acid-like oligomers, which are targeted to a nucleic acid encoding human Dicer, and which act to modulate the levels of the human Dicer RNase III enzyme and interfere with its function, as well as modulating the levels of small non-coding RNAs.

Pharmaceutical and other compositions comprising the compounds of the invention are also provided.

Also provided are methods of screening for modulators of small non-coding RNAs and methods of modulating the levels of small non-coding RNAs in cells, tissues or animals comprising contacting said cells, tissues or animals with one or more of the compounds or compositions of the invention.

Methods of treating an animal, particularly a human, suspected of having or being prone to a disease or condition associated with expression of small non-coding RNAs are also set forth herein. Such methods comprise optionally identifying such an animal, and administering a therapeutically or prophylactically effective amount of one or more of the compounds or compositions of the invention to the animal or person.

Brief Description of the Drawings

Figure 1 shows the interaction of the mir-143 miRNA with three novel binding sites in the ERK5 mRNA coding sequence (GenBank Accession NM_139032.1) identified herein, along with their bimolecular hybridization free energies.

Detailed Description of the Invention

The present invention provides oligomeric compounds useful in, for example, the modulation of expression, endogenous levels or the function of small non-coding RNAs. As used herein, the term "small non-coding RNA" is used to encompass, without limitation, a polynucleotide molecule ranging from about 17 to about 450 nucleotides in length, which can be endogenously transcribed or produced exogenously (chemically or synthetically), but is not translated into a protein. Small non-coding RNAs may include isolated single-, double-, or multiple-stranded molecules, any of which may include regions of intrastrand nucleobase complementarity, said regions capable of folding and forming a molecule with fully or partially double-stranded or multiple-stranded character based on regions of perfect or imperfect complementarity. Examples of small non-coding RNAs include, but are not limited to, primary miRNA transcripts (also known as pri-pre-miRNAs, pri-mirs and pri-miRNAs, which range from around 70 nucleotides to about 450 nucleotides in length and often taking the form of a hairpin structure); pre-miRNAs (also known as pre-mirs and foldback miRNA precursors, which range from around 50 nucleotides to around 110 nucleotides in length); miRNAs (also known as microRNAs, Mirs, miRs, mirs, and mature miRNAs, and generally refer either to double-stranded intermediate molecules around 17 to about 25 nucleotides in length, or to single-stranded miRNAs, which may comprise a bulged structure upon hybridization with a partially

complementary target nucleic acid molecule); or mimics of pri-miRNAs, pre-miRNAs or miRNAs. Small non-coding RNAs can be endogenously transcribed in cells, or can be synthetic oligonucleotides, *in vitro* transcribed polynucleotides or nucleic acid oligomeric compounds expressed from vectors. Pri-miRNAs and pre-miRNAs, or mimics thereof, may be processed into smaller molecules.

As used herein, the term "miRNA precursor" is used to encompass, without limitation, primary RNA transcripts, pri-miRNAs and pre-miRNAs.

In some embodiments, pri-miRNAs, or mimics thereof, are 70 to 450 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449 or 450 nucleobases in length, or any range therewithin.

In some embodiments, pri-miRNAs, or mimics thereof, are 110 to 430 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125,

126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429 or 430 nucleobases in length, or any range therewithin.

In some embodiments, pri-miRNAs, or mimics thereof, are 110 to 280 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279 or 280 nucleobases in length, or any range therewithin.

In some embodiments, pre-miRNAs, or mimics thereof, are 50 to 110 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69 70, 71 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97,

98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109 or 110 nucleobases in length, or any range therewithin. In some embodiments, pre-miRNAs, or mimics thereof, are 60 to 80 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 nucleobases in length, or any range therewithin.

In some embodiments, miRNAs, or mimics thereof, are 15 to 49 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 or 49 nucleobases in length, or any range therewithin. In some embodiments, miRNAs, or mimics thereof, are 17 to 25 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 17, 18, 19, 20, 21, 22, 23, 24 or 25 nucleobases in length, or any range therewithin.

Oligomeric compounds of the invention modulate the levels, expression or function of small non-coding RNAs by hybridizing to a nucleic acid comprising or encoding a small non-coding RNA nucleic acid target resulting in alteration of normal function by, for example, facilitating destruction of the small non-coding RNA through cleavage, by sequestration, or by sterically occluding the function of the small non-coding RNA. Further, modified synthetic oligomeric compounds of the present invention may be designed to mimic endogenous small non-coding RNAs. These modifications include, but are not limited to, improved pharmacokinetic or pharmacodynamic properties, binding affinity, stability, charge, localization or uptake. Synthetic mimics can therefore act as replacements for small non-coding RNAs, as competitive inhibitors of naturally occuring small non-coding RNAs or as delivery systems wherein the mimic construct contains one or more functional components.

As used herein, the terms "target nucleic acid," "target RNA," "target RNA transcript" or "nucleic acid target" are used to encompass any nucleic acid capable of being targeted including, without limitation, RNA (including microRNAs, stRNAs, small nuclear RNAs, small nucleolar RNAs, small ribosomal RNAs, small hairpin RNAs, endogenous antisense RNAs, guide RNAs, tiny noncoding RNAs, small single or double stranded RNAs that are encoded by heterochromatic repeats at centromeres or other chromosomal origin, and any precursors thereof). These nucleic acid targets can be coding or non-coding sequences; pre-mRNAs or mRNAs; single- or double-stranded, or single-stranded with partial double-stranded character; may occur naturally within introns or exons of messenger RNAs (mRNAs), ribosomal RNAs

(rRNAs), or transfer RNAs (tRNAs); and can be endogenously transcribed or exogenously produced.

In some embodiments of this invention, modulation of small non-coding RNA levels, expression or function is achieved via oligomeric compounds which target a further RNA associated with the particular small non-coding RNA. This association can be a physical association between that RNA and the particular small non-coding RNA such as, but not limited to, in an RNA or ribonucleoprotein complex. This association can also be within the context of a biological pathway, such as but not limited to, the regulation of levels, expression or function of a protein-encoding mRNA or its precursor by a small non-coding RNA. As such, the invention provides for modulation of the levels, expression or function of a target nucleic acid where the target nucleic acid is a messenger RNA whose expression levels and/or function are associated with one or more small non-coding RNAs. The messenger RNA function or processing may be disrupted by degradation through an antisense mechanism, including but not limited to, RNA interference, or RNase H, as well as other mechanisms wherein double stranded nucleic acid structures are recognized and degraded, cleaved, sterically occluded, sequestered or otherwise rendered inoperable.

The compounds or compositions of the present invention may also interfere with the function of endogenous RNA molecules. The functions of RNA to be interfered with can include, for example, nuclear events such as replication or transcription as the compounds of the present invention could target or mimic small non-coding RNAs in these cellular processes. Replication and transcription, for example, can be from an endogenous cellular template, a vector, a plasmid construct or otherwise. The functions of RNA to be interfered with can include cytoplasmic events such as translocation of the RNA to a site of protein translation, translocation of the RNA to sites within the cell which are distant from the site of RNA synthesis, translation of protein from the RNA, splicing of the RNA to yield one or more RNA species, RNA signaling and regulatory activities, and catalytic activity or complex formation involving the RNA which may be engaged in or facilitated by the RNA as the compounds of the present invention could target or mimic small non-coding RNAs in these cellular processes.

In the context of the present invention, "modulation" and "modulation of expression" mean either an increase (stimulation) or a decrease (inhibition) in the amount or levels of a small non-coding RNA, nucleic acid target, an RNA or protein associated with a small non-coding RNA, or a downstream target of the small non-coding RNA (e.g., a mRNA representing a protein-coding nucleic acid that is regulated by a small non-coding RNA). Inhibition is a suitable form of modulation and small non-coding RNA is a suitable target nucleic acid.

In the context of the present invention, "modulation of function" means an alteration in the function of the small non-coding RNA or an alteration in the function of any cellular component with which the small non-coding RNA has an association or downstream effect.

The present invention provides, *inter alia*, oligomeric compounds and compositions containing the same wherein the oligomeric compound includes one or more modifications that render the compound capable of supporting modulation of the levels, expression or function of the small non-coding RNA by a degradation or cleavage mechanism.

The present invention also provides methods of maintaining a pluripotent stem cell comprising contacting the cell with an effective amount of an oligomeric compound targeting human Dicer. The pluripotent stem cell can be present in a sample of cord blood or bone marrow, or may be present as part of a cell line. In addition, the pluripotent stem cell can be an embryonic stem cell.

The present invention also provides oligomeric compounds and compositions containing the same wherein the oligomeric compound includes one or more modifications that render the compound capable of blocking or interfering with the levels, expression or function of one or more small non-coding RNAs by steric occlusion.

The present invention also provides oligomeric compounds and compositions containing the same wherein the oligomeric compound includes one or more modifications or structural elements or motifs that render the compound capable of mimicking or replacing one or more small non-coding RNAs.

Oligomeric Compounds

In the context of the present invention, the term "oligomeric compound(s)" refers to polymeric structures which are capable of hybridizing to at least a region of a small non-coding RNA molecule or a target of small non-coding RNAs, or polymeric structures which are capable of mimicking small non-coding RNAs. The term "oligomeric compound" includes, but is not limited to, compounds comprising oligonucleotides, oligonucleosides, oligonucleotide analogs, oligonucleotide mimetics and combinations of these. Oligomeric compounds also include, but are not limited to, antisense oligomeric compounds, antisense oligonucleotides, siRNAs, alternate splicers, primers, probes and other compounds that hybridize to at least a portion of the target nucleic acid. Oligomeric compounds are routinely prepared linearly but can be joined or otherwise prepared to be circular and may also include branching. Separate oligomeric compounds can hybridize to form double stranded compounds that can be blunt-ended or may include overhangs on one or both termini. In general, an oligomeric compound comprises a backbone of linked monomeric subunits where each linked monomeric subunit is directly or

indirectly attached to a heterocyclic base moiety. The linkages joining the monomeric subunits, the sugar moieties or sugar surrogates and the heterocyclic base moieties can be independently modified giving rise to a plurality of motifs for the resulting oligomeric compounds including hemimers, gapmers and chimeras.

As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base moiety. The two most common classes of such heterocyclic bases are purines and pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. The respective ends of this linear polymeric structure can be joined to form a circular structure by hybridization or by formation of a covalent bond. In addition, linear compounds may have internal nucleobase complementarity and may therefore fold in a manner as to produce a fully or partially double-stranded structure. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide. The normal internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage.

In the context of this invention, the term "oligonucleotide" refers generally to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). This term includes oligonucleotides composed of naturally occurring nucleobases, sugars and covalent internucleoside linkages. The term "oligonucleotide analog" refers to oligonucleotides that have one or more non-naturally occurring portions which function in a similar manner to oligonucleotides. Such non-naturally occurring oligonucleotides are often selected over naturally occurring forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for other oligonucleotides or nucleic acid targets and increased stability in the presence of nucleases.

In the context of this invention, the term "oligonucleoside" refers to nucleosides that are joined by internucleoside linkages that do not have phosphorus atoms. Internucleoside linkages of this type include short chain alkyl, cycloalkyl, mixed heteroatom alkyl, mixed heteroatom cycloalkyl, one or more short chain heteroatomic and one or more short chain heterocyclic. These internucleoside linkages include but are not limited to siloxane, sulfide, sulfoxide, sulfone, acetyl, formacetyl, thioformacetyl, methylene formacetyl, thioformacetyl, alkeneyl, sulfamate; methyleneimino, methylenehydrazino, sulfonate, sulfonamide, amide and others having mixed N, O, S and CH_2 component parts. In addition to the modifications described above, the

nucleosides of the oligomeric compounds of the invention can have a variety of other modifications. Additional nucleosides amenable to the present invention having altered base moieties and or altered sugar moieties are disclosed in U.S. Patent 3,687,808 and PCT application PCT/US89/02323.

For nucleotides that are incorporated into oligonucleotides of the invention, these nucleotides can have sugar portions that correspond to naturally occurring sugars or modified sugars. Representative modified sugars include carbocyclic or acyclic sugars, sugars having substituent groups at one or more of their 2', 3' or 4' positions and sugars having substituents in place of one or more hydrogen atoms of the sugar.

Altered base moieties or altered sugar moieties also include other modifications consistent with the spirit of this invention. Such oligomeric compounds are best described as being structurally distinguishable from, yet functionally interchangeable with, naturally occurring or synthetic unmodified oligonucleotides. All such oligomeric compounds are comprehended by this invention so long as they function effectively to mimic the structure or function of a desired RNA or DNA oligonucleotide strand.

A class of representative base modifications include tricyclic cytosine analog, termed "G clamp" (Lin, *et al.*, *J. Am. Chem. Soc.* 1998, 120, 8531). This analog can form four hydrogen bonds with a complementary guanine (G) by simultaneously recognizing the Watson-Crick and Hoogsteen faces of the targeted G. This G clamp modification when incorporated into phosphorothioate oligomeric compounds, dramatically enhances potencies as measured by target reduction in cell culture. The oligomeric compounds of the invention also can include phenoxazine-substituted bases of the type disclosed by Flanagan, et al., Nat. Biotechnol. 1999, 17(1), 48-52.

The oligomeric compounds in accordance with this invention comprise from about 8 to about 80 monomeric subunits (i.e. from about 8 to about 80 linked nucleosides). One of ordinary skill in the art will appreciate that the invention embodies oligomeric compounds of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 subunits in length, or any range therewithin.

In one embodiment, the oligomeric compounds of the invention are 12 to 50 monomeric subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30,

31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 subunits in length, or any range therewithin.

In one embodiment, the oligomeric compounds of the invention are 13 to 80 monomeric subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 subunits in length, or any range therewithin.

In one embodiment, the oligomeric compounds of the invention are 15 to 30 monomeric subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 subunits in length, or any range therewithin.

In one embodiment, the oligomeric compounds of the invention are 70 to 450 monomeric subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431,

432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449 or 450 subunits in length, or any range therewithin.

In one embodiment, the oligomeric compounds of the invention are 110 to 430 monomeric subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429 or 430 subunits in length, or any range therewithin.

In one embodiment, the oligomeric compounds of the invention are 110 to 280 monomeric subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254,

255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279 or 280 subunits in length, or any range therewithin.

In one embodiment, the oligomeric compounds of the invention are 50 to 110 monomeric subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69 70, 71 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109 or 110 subunits in length, or any range therewithin.

In one embodiment, the oligomeric compounds of the invention are 60 to 80 monomeric subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 subunits in length, or any range therewithin.

In one embodiment, the oligomeric compounds of the invention are 15 to 49 monomeric subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 or 49 subunits in length, or any range therewithin.

In one embodiment, the oligomeric compounds of the invention are 17 to 25 subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 17, 18, 19, 20, 21, 22, 23, 24 or 25 subunits in length, or any range therewithin.

In accordance with the present invention, oligomeric compounds designed to mimic pri-miRNAs are from about 70 to about 450 monomeric subunits in length, or from about 110 to 430 subunits in length. Oligomeric compounds of the invention designed to mimic pre-miRNAs are from about 50 to about 110 monomeric subunits in length, or from about 60 to about 80 subunits in length. Oligomeric compounds of the invention designed to mimic mature miRNAs are from about 17 to about 25 monomeric subunits in length, and can be single- or double-stranded with either or both strands comprising from about 17 to about 25 subunits.

As used herein, the term "about" means ± 5% of the variable thereafter.

The size or length of any oligomeric compound of the present invention, within any range cited herein, can be determined as follows:

Let R(n, n+m-1) be a region from a target nucleobase sequence, where "n" is the 5'-most nucleobase position of the region, where "n+m-1" is the 3'-most nucleobase position of the

region and where "m" is the length of the region. A set "S(m)", of regions of length "m" is defined as the regions where n ranges from 1 to L-m+1, where L is the length of the target nucleic acid sequence and L>m. A set, "A", of all regions can be constructed as a union of the sets of regions for each length from where m is greater than or equal to a lower limit of any recited range (8 in this example) and is less than or equal to the upper limit of any recited range (80 in this example).

This set of regions can be represented using the following mathematical notation:

$$A = \bigcup_{m} S(m) \quad \text{where } m \in N | 8 \leq m \leq 80$$

and

$$S(m) = \{R_{n,n+m-1} | n \in \{1,2,3,\ldots, L-m+1\}\}$$

where the mathematical operator | indicates "such that",

where the mathematical operator $\in$ indicates "a member of a set" (e.g. y $\in$ Z indicates that element y is a member of set Z),

where x is a variable,

where N indicates all natural numbers, defined as positive integers,

and where the mathematical operator $\bigcup$ indicates "the union of sets".

For example, the set of regions for m equal to 8, 20 and 80 can be constructed in the following manner. The set of regions, each 8 monomeric subunits in length, S(m=8), in a target nucleic acid sequence 100 subunits in length (L=100), beginning at position 1 (n=1) of the target nucleic acid sequence, can be created using the following expression:

$$S(8) = \{R_{1,8} | n \in \{1,2,3,\ldots,93\}\}$$

and describes the set of regions comprising nucleobases 1-8, 2-9, 3-10, 4-11, 5-12, 6-13, 7-14, 8-15, 9-16, 10-17, 11-18, 12-19, 13-20, 14-21, 15-22, 16-23, 17-24, 18-25, 19-26, 20-27, 21-28, 22-29, 23-30, 24-31, 25-32, 26-33, 27-34, 28-35, 29-36, 30-37, 31-38, 32-39, 33-40, 34-41, 35-42, 36-43, 37-44, 38-45, 39-46, 40-47, 41-48, 42-49, 43-50, 44-51, 45-52, 46-53, 47-54, 48-55, 49-56, 50-57, 51-58, 52-59, 53-60, 54-61, 55-62, 56-63, 57-64, 58-65, 59-66, 60-67, 61-68, 62-69, 63-70, 64-71, 65-72, 66-73, 67-74, 68-75, 69-76, 70-77, 71-78, 72-79, 73-80, 74-81, 75-82, 76-83, 77-84, 78-85, 79-86, 80-87, 81-88, 82-89, 83-90, 84-91, 85-92, 86-93, 87-94, 88-95, 89-96, 90-97, 91-98, 92-99, 93-100.

An additional set for regions 20 monomeric subunits in length, in a target sequence 100 subunits in length, beginning at position 1 of the target nucleic acid sequence, can be described using the following expression:

$$S(20) = \{R_{1,20} | n \in \{1,2,3,...,81\}\}$$

and describes the set of regions comprising nucleobases 1-20, 2-21, 3-22, 4-23, 5-24, 6-25, 7-26, 8-27, 9-28, 10-29, 11-30, 12-31, 13-32, 14-33, 15-34, 16-35, 17-36, 18-37, 19-38, 20-39, 21-40, 22-41, 23-42, 24-43, 25-44, 26-45, 27-46, 28-47, 29-48, 30-49, 31-50, 32-51, 33-52, 34-53, 35-54, 36-55, 37-56, 38-57, 39-58, 40-59, 41-60, 42-61, 43-62, 44-63, 45-64, 46-65, 47-66, 48-67, 49-68, 50-69, 51-70, 52-71, 53-72, 54-73, 55-74, 56-75, 57-76, 58-77, 59-78, 60-79, 61-80, 62-81, 63-82, 64-83, 65-84, 66-85, 67-86, 68-87, 69-88, 70-89, 71-90, 72-91, 73-92, 74-93, 75-94, 76-95, 77-96, 78-97, 79-98, 80-99, 81-100.

An additional set for regions 80 monomeric subunits in length, in a target sequence 100 subunits in length, beginning at position 1 of the target nucleic acid sequence, can be described using the following expression:

$$S(80) = \{R_{1,80} | n \in \{1,2,3,...,21\}\}$$

and describes the set of regions comprising nucleobases 1-80, 2-81, 3-82, 4-83, 5-84, 6-85, 7-86, 8-87, 9-88, 10-89, 11-90, 12-91, 13-92, 14-93, 15-94, 16-95, 17-96, 18-97, 19-98, 20-99, 21-100.

The union of these aforementioned example sets and other sets for lengths from 10 to 19 and 21 to 79 can be described using the mathematical expression

$$A = \bigcup_{m} S(m)$$

where $\bigcup$ represents the union of the sets obtained by combining all members of all sets.

Thus, in this example, A would include regions 1-8, 2-9, 3-10...93-100, 1-20, 2-21, 3-22...81-100, 1-80, 2-81, 3-82...21-100.

The mathematical expressions described herein define all possible target regions in a target nucleic acid sequence of any length L, where the region is of length m, and where m is greater than or equal to the lower limit and less than or equal to the upper limit of monomeric units, and where m is less than L, and where n is less than L-m+1.

In the context of this invention, "hybridization" means the pairing of complementary strands of oligomeric compounds. In the present invention, the mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases (nucleobases) of the strands of oligomeric compounds. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds. Hybridization can occur under varying circumstances.

An oligomeric compound of the invention is "specifically hybridizable" when association of the compound with the target nucleic acid interferes with the normal function of the target nucleic acid to alter the activity, disrupt the function, or modulate the level of the target nucleic acid, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligomeric compound to non-target nucleic acid sequences under conditions in which specific hybridization is desired, i.e., under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and under standard assay conditions in the case of *in vitro* assays.

In the present invention the phrase "stringent hybridization conditions" or "stringent conditions" refers to conditions under which an oligomeric compound of the invention will hybridize to its target sequence, but to a minimal number of other sequences. Stringent conditions are sequence-dependent and will vary with different circumstances and in the context of this invention; "stringent conditions" under which oligomeric compounds hybridize to a target sequence are determined by the nature and composition of the oligomeric compounds and the assays in which they are being investigated. One having ordinary skill in the art will understand variability in the experimental protocols and be able to determine when conditions are optimal for stringent hybridization with minimal non-specific hybridization events.

"Complementary," as used herein, refers to the capacity for precise pairing of two monomeric subunits regardless of where in the oligomeric compound or target nucleic acid the two are located. For example, if a monomeric subunit at a certain position of an oligomeric compound is capable of hydrogen bonding with a monomeric subunit at a certain position of a target nucleic acid, then the position of hydrogen bonding between the oligomeric compound and the target nucleic acid is considered to be a complementary position. The oligomeric compound and the target nucleic acid are "substantially complementary" to each other when a sufficient number of complementary positions in each molecule are occupied by monomeric subunits that can hydrogen bond with each other. Thus, the term "substantially complementary" is used to indicate a sufficient degree of precise pairing over a sufficient number of monomeric subunits such that stable and specific binding occurs between the oligomeric compound and a target nucleic acid.

Generally, an oligomeric compound is "antisense" to a target nucleic acid when, written in the 5' to 3' direction, it comprises the reverse complement of the corresponding region of the target nucleic acid. "Antisense compounds" are also often defined in the art to comprise the further limitation of, once hybridized to a target, being able to induce or trigger a reduction in target gene expression.

It is understood in the art that the sequence of the oligomeric compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. Moreover, an oligomeric compound may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization (e.g., a bulge, a loop structure or a hairpin structure).

In some embodiments of the invention, the oligomeric compounds comprise at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, or at least 85% sequence complementarity to a target region within the target nucleic acid. In other embodiments of the invention, the oligomeric compounds comprise at least 90% sequence complementarity to a target region within the target nucleic acid. In other embodiments of the invention, the oligomeric compounds comprise at least 95% or at least 99% sequence complementarity to a target region within the target nucleic acid. For example, an oligomeric compound in which 18 of 20 nucleobases of the oligomeric compound are complementary to a target sequence would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an oligomeric compound which is 18 nucleobases in length having 4 (four) noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an oligomeric compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul et al., J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656).

In some embodiments of the invention, the oligomeric compounds act as mimics or replacements for small non-coding RNAs. In this case, the oligomeric compounds of the invention can comprise at least 70% sequence identity to a small non-coding RNA or a region thereof. In some embodiments the oligomeric compounds of the invention can comprise at least 90% sequence identity and in some embodiments can comprise at least 95% sequence identity to to a small non-coding RNA or a region thereof.

"Targeting" an oligomeric compound to a particular nucleic acid molecule, in the context of this invention, can be a multistep process. The process usually begins with the identification of a target nucleic acid whose levels, expression or function is to be modulated. This target nucleic acid may be, for example, a mRNA transcribed from a cellular gene whose

expression is associated with a particular disorder or disease state, a small non-coding RNA or its precursor, or a nucleic acid molecule from an infectious agent.

The targeting process usually also includes determination of at least one target region, segment, or site within the target nucleic acid for the interaction to occur such that the desired effect, e.g., modulation of levels, expression or function, will result. Within the context of the present invention, the term "region" is defined as a portion of the target nucleic acid having at least one identifiable sequence, structure, function, or characteristic. Within regions of target nucleic acids are segments. "Segments" are defined as smaller or sub-portions of regions within a target nucleic acid. "Sites," as used in the present invention, are defined as specific positions within a target nucleic acid. The terms region, segment, and site can also be used to describe an oligomeric compound of the invention such as for example a gapped oligomeric compound having three separate segments.

Targets of the present invention include both coding and non-coding nucleic acid sequences. For coding nucleic acid sequences, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon." A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function *in vivo*. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used *in vivo* to initiate translation of an mRNA transcribed from a gene encoding a nucleic acid target, regardless of the sequence(s) of such codons. It is also known in the art that a translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively).

The terms "start codon region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either

direction (i.e., 5' or 3') from a translation termination codon. Consequently, the "start codon region" (or "translation initiation codon region") and the "stop codon region" (or "translation termination codon region") are all regions which may be targeted effectively with the oligomeric compounds of the present invention.

The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Within the context of the present invention, a further suitable region is the intragenic region encompassing the translation initiation or termination codon of the open reading frame (ORF) of a gene.

Other target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA (or corresponding nucleotides on the gene), and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA (or corresponding nucleotides on the gene). The 5' cap site of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap site. It is also suitable to target the 5' cap region.

Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. Targeting splice sites, i.e., intron-exon junctions or exon-intron junctions, may also be particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also target sites. mRNA transcripts produced via the process of splicing of two (or more) mRNAs from different gene sources are known as "fusion transcripts." It is also known that introns can be effectively targeted using oligomeric compounds targeted to, precursor molecules for example, pre-mRNA.

It is also known in the art that alternative RNA transcripts can be produced from the same genomic region of DNA. These alternative transcripts are generally known as "variants." More specifically, "pre-mRNA variants" are transcripts produced from the same genomic DNA that differ from other transcripts produced from the same genomic DNA in either their start or stop position and contain both intronic and exonic sequences.

Upon excision of one or more exon or intron regions, or portions thereof, during splicing, pre-mRNA variants produce smaller "mRNA variants." Consequently, mRNA variants are processed pre-mRNA variants and each unique pre-mRNA variant must always produce a unique mRNA variant as a result of splicing. These mRNA variants are also known as "alternative splice variants." If no splicing of the pre-mRNA variant occurs then the pre-mRNA variant is identical to the mRNA variant.

It is also known in the art that variants can be produced through the use of alternative signals to start or stop transcription and that pre-mRNAs and mRNAs can possess more that one start codon or stop codon. Variants that originate from a pre-mRNA or mRNA that use alternative start codons are known as "alternative start variants" of that pre-mRNA or mRNA. Those transcripts that use an alternative stop codon are known as "alternative stop variants" of that pre-mRNA or mRNA. One specific type of alternative stop variant is the "polyA variant" in which the multiple transcripts produced result from the alternative selection of one of the "polyA stop signals" by the transcription machinery, thereby producing transcripts that terminate at unique polyA sites. Within the context of the invention, the types of variants described herein are also target nucleic acids.

Certain non-coding RNA genes are known to produce functional RNA molecules with important roles in diverse cellular processes. Such non-translated, non-coding RNA molecules can include ribosomal RNAs, tRNAs, snRNAs, snoRNAs, tncRNAs, rasiRNAs, short hairpin RNAs (shRNAs), short temporal RNAs (stRNAs), short hairpin RNAs (shRNAs), siRNAs, miRNAs and smnRNAs. These non-coding RNA genes and their products are also suitable targets of the compounds of the invention. Such cellular processes include transcriptional regulation, translational regulation, developmental timing, viral surveillance, immunity, chromosome maintenance, ribosomal structure and function, gene imprinting, subcellular compartmentalization, pre-mRNA splicing, and guidance of RNA modifications. RNA-mediated processes are now also believed to direct heterochromatin formation, genome rearrangements, cellular differentiation and DNA elimination.

A total of 201 different expressed RNA sequences potentially encoding novel small non-messenger species (smnRNAs) has been identified from mouse brain cDNA libraries. Based on sequence and structural motifs, several of these have been assigned to the snoRNA class of nucleolar localized molecules known to act as guide RNAs for rRNA modification, whereas others are predicted to direct modification within the U2, U4, or U6 small nuclear RNAs (snRNAs). Some of these newly identified smnRNAs remained unclassified and have no identified RNA targets. It was suggested that some of these RNA species may have novel

functions previously unknown for snoRNAs, namely the regulation of gene expression by binding to and/or modifying mRNAs or their precursors via their antisense elements (Huttenhofer et al., Embo J., 2001, 20, 2943-2953). Therefore, these smnRNAs are also suitable targets for the compounds of the present invention.

The locations on the target nucleic acid to which compounds and compositions of the invention hybridize are herein referred to as "suitable target segments." As used herein the term "suitable target segment" is defined as at least an 8-nucleobase portion of a target region to which oligomeric compound is targeted.

Once one or more targets, target regions, segments or sites have been identified, oligomeric compounds are designed to be sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect. The desired effect may include, but is not limited to modulation of the levels, expression or function of the target.

In accordance with the present invention, a series of single stranded oligomeric compounds can be designed to target or mimic one or more specific small non-coding RNAs. These oligomeric compounds can be of a specified length, for example from 8 to 80, 12 to 50, 13 to 80, 15 to 30, 70 to 450, 110 to 430, 110 to 280, 50 to 110, 60 to 80, 15 to 49, 17 to 25 or 19 to 23 nucleotides long and have one or more modifications.

In accordance with one embodiment of the invention, a series of double-stranded oligomeric compounds (duplexes) comprising, as the antisense strand, the single-stranded oligomeric compounds of the present invention, and the fully or partially complementary sense strand, can be designed to modulate the levels, expression or function of one or more small non-coding RNAs or small non-coding RNA targets. One or both termini of the duplex strands may be modified by the addition of one or more natural or modified nucleobases to form an overhang. The sense strand of the duplex may be designed and synthesized as the complement of the antisense strand and may also contain modifications or additions to either terminus. For example, in one embodiment, both strands of the duplex would be complementary over the central region of the duplex, each having overhangs at one or both termini.

For the purposes of this invention, the combination of an antisense strand and a sense strand, each of which can be of a specified length, for example from 8 to 80, 12 to 50, 13 to 80, 15 to 30, 15 to 49, 17 to 25 or 19 to 23 subunits long, is identified as a complementary pair of oligomeric compounds. This complementary pair of oligonucleotides can include additional nucleotides on either of their 5' or 3' ends. They can include other molecules or molecular structures on their 3' or 5' ends, such as a phosphate group on the 5' end, or non-nucleic acid

moieties conjugated to either terminus of either strand or both strands. One group of compounds of the invention includes a phosphate group on the 5' end of the antisense strand compound. Other compounds also include a phosphate group on the 5' end of the sense strand compound. Some compounds include additional nucleotides such as a two base overhang on the 3' end as well as those lacking overhangs.

For example, a complementary pair of oligomeric compounds may comprise an antisense strand oligomeric compound having the sequence CGAGAGGCGGACGGGACCG (SEQ ID NO:2181), having a two-nucleobase overhang of deoxythymidine (dT) and its complement sense strand. This complementary pair of oligomeric compounds would have the following structure:

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  cgagaggcggacgggaccgTT      Antisense  Strand (SEQ ID NO:2182)
  ||||||||||||||||||||
TTgctctccgcctgccctggc       Complement Sense Strand (SEQ ID NO:2183)
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In some embodiments, a single-stranded oligomeric compound may be designed comprising the antisense portion as a first region and the sense portion as a second region. The first and second regions can be linked together by either a nucleotide linker (a string of one or more nucleotides that are linked together in a sequence) or by a non-nucleotide linker region or by a combination of both a nucleotide and non-nucleotide structure. In any of these structures, the oligomeric compound, when folded back on itself, would form at least a partially complementary structure at least between a portion of the first region, the antisense portion, and a portion of the second region, the sense portion.

In one embodiment, the invention includes an oligomeric compound/protein composition. This composition has both an oligomeric compound component and a protein component. The oligomeric compound component comprises at least one oligomeric compound, either the antisense or the sense oligomeric compound but preferably the antisense oligomeric compound (the oligomeric compound that is antisense to the target nucleic acid). The protein component of the composition comprises at least one protein that forms a portion of the RNA-induced silencing complex, i.e., the RISC complex. The oligomeric compound component can also comprise both antisense and sense strand oligomeric compounds.

RISC is a ribonucleoprotein complex that contains proteins of the Argonaute family of proteins. While not wishing to be bound by theory, it is believed that the Argonaute proteins are a class of proteins, some of which have been shown to contain a PAZ and/or a Piwi domain and that have been implicated in processes previously linked to posttranscriptional silencing. The Argonaute family of proteins includes, but depending on species, is not necessary limited to

elF2C1 and elF2C2. It is also believed that at least the antisense strand of double-stranded compounds shown to act as siRNAs is bound to one of the protein components that form the RISC complex, and that the RISC complex interacts with the ribosomes or polyribosome complexes which may contain small non-coding RNA molecules amenable to targeting with the oligomeric compounds of the present invention. Consequently, one embodiment of the invention includes oligomeric compounds that mimic RNA components of the RISC complex.

In one embodiment, the oligomeric compounds of the invention are designed to exert their modulatory effects via mimicking or targeting small non-coding RNAs associated with cellular factors such as transporters or chaperones. These cellular factors can be protein, lipid or carbohydrate based and can have structural or enzymatic functions that may or may not require the complexation of one or more metal ions.

Furthermore, the oligomeric compounds of the invention can have one or more moieties bound or conjugated, which facilitates the active or passive transport, localization, or compartmentalization of the oligomeric compound. Cellular localization includes, but is not limited to, localization to within the nucleus, the nucleolus, or the cytoplasm. Compartmentalization includes, but is not limited to, any directed movement of the oligonucleotides of the invention to a cellular compartment including the nucleus, nucleolus, mitochondrion, or imbedding into a cellular membrane.

In some embodiments of the invention, the oligomeric compounds are designed to exert their modulatory effects via mimicking or targeting small non-coding RNAs associated with cellular factors that affect gene expression, more specifically those involved in RNA or DNA modifications. These modifications include, but are not limited to, posttranscriptional or chromosomal modifications such as methylation, acetylation, pseudouridylation or amination.

Furthermore, the oligomeric compounds of the invention comprise one or more conjugate moieties which facilitate posttranscriptional modification.

The oligomeric compounds of the invention may be in the form of single-stranded, double-stranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges or loops. Once introduced to a system, the oligomeric compounds of the invention may elicit the action of one or more enzymes or proteins to effect modulation of the levels, expression or function of the target nucleic acid.

One non-limiting example of such a protein is the Drosha RNase III enzyme. Drosha is a nuclear enzyme that processes long primary RNA transcripts (pri-miRNAs) from approximately 70 to 450 nucleotides in length into pre-miRNAs (from about 50 to about 80 nucleotides in length) which are exported from the nucleus to encounter the human Dicer

enzyme which then processes pre-miRNAs into miRNAs. It is believed that, in processing the pri-miRNA into the pre-miRNA, the Drosha enzyme cuts the pri-miRNA at the base of the mature miRNA, leaving a 2-nt 3'overhang (Lee, et al., Nature, 2003, 425, 415-419). The 3' two-nucleotide overhang structure, a signature of RNaseIII enzymatic cleavage, has been identified as a critical specificity determinant in targeting and maintaining small RNAs in the RNA interference pathway (Murchison, et al., Curr. Opin. Cell Biol., 2004, 16, 223-9).

A further non-limiting example involves the enzymes of the RISC complex. Use of the RISC complex to effect cleavage of RNA targets thereby greatly enhances the efficiency of oligonucleotide-mediated inhibition of gene expression. Similar roles have been postulated for other ribonucleases such as those in the RNase III and ribonuclease L family of enzymes.

Oligomeric compounds or compositions of the invention are used to induce potent and specific modulation of gene function through interactions with or mimicry of small non-coding RNAs that are processed by the RISC complex. These compounds include single-stranded oligomeric compounds that bind in a RISC complex, double-stranded antisense/sense pairs of oligomeric compounds, or single-stranded oligomeric compounds that include both an antisense portion and a sense portion.

General Oligomer Synthesis:

Oligomerization of modified and unmodified nucleosides is performed according to literature procedures for DNA like compounds (Protocols for Oligonucleotides and Analogs, Ed. Agrawal (1993), Humana Press) and/or RNA like compounds (Scaringe, Methods (2001), 23, 206-217. Gait et al., Applications of Chemically synthesized RNA in RNA:Protein Interactions, Ed. Smith (1998), 1-36. Gallo et al., Tetrahedron (2001), 57, 5707-5713) synthesis as appropriate. In addition, specific protocols for the synthesis of oligomeric compounds of the invention are illustrated in the examples below.

RNA oligomers can be synthesized by methods disclosed herein or purchased from various RNA synthesis companies such as for example Dharmacon Research Inc., (Lafayette, CO).

Irrespective of the particular protocol used, the oligomeric compounds used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed.

Synthesis of Nucleoside Phosphoramidites:

The following compounds, including amidites and their intermediates were prepared as described in US Patent 6,426,220 and published PCT WO 02/36743; 5'-O-Dimethoxytrityl-thymidine intermediate for 5-methyl dC amidite, 5'-O-Dimethoxytrityl-2'-deoxy-5-methylcytidine intermediate for 5-methyl-dC amidite, 5'-O-Dimethoxytrityl-2'-deoxy-N4-benzoyl-5-methylcytidine penultimate intermediate for 5-methyl dC amidite, (5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-2'-deoxy-N^4-benzoyl-5-methylcytidin-3'-*O*-yl)-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (5-methyl dC amidite), 2'-Fluorodeoxyadenosine, 2'-Fluorodeoxyguanosine, 2'-Fluorouridine, 2'-Fluorodeoxycytidine, 2'-O-(2-Methoxyethyl) modified amidites, 2'-O-(2-methoxyethyl)-5-methyluridine intermediate, 5'-O-DMT-2'-O-(2-methoxyethyl)-5-methyluridine penultimate intermediate, (5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-2'-*O*-(2-methoxyethyl)-5-methyluridin-3'-*O*-yl)-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (MOE T amidite), 5'-O-Dimethoxytrityl-2'-O-(2-methoxyethyl)-5-methylcytidine intermediate, 5'-O-dimethoxytrityl-2'-*O*-(2-methoxyethyl)-N^4-benzoyl-5-methyl-cytidine penultimate intermediate, (5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-2'-*O*-(2-methoxyethyl)-N^4-benzoyl-5-methylcytidin-3'-*O*-yl)-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (MOE 5-Me-C amidite), (5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-2'-*O*-(2-methoxyethyl)-N^6-benzoyladenosin-3'-*O*-yl)-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (MOE A amdite), (5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-2'-*O*-(2-methoxyethyl)-N^4-isobutyrylguanosin-3'-*O*-yl)-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (MOE G amidite), 2'-O-(Aminooxyethyl) nucleoside amidites and 2'-O-(dimethylaminooxyethyl) nucleoside amidites, 2'-(Dimethylaminooxyethoxy) nucleoside amidites, 5'-O-tert-Butyldiphenylsilyl-O^2-2'-anhydro-5-methyluridine , 5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine, 2'-O-((2-phthalimidoxy)ethyl)-5'-*t*-butyldiphenylsilyl-5-methyluridine , 5'-O-*tert*-butyldiphenylsilyl-2'-O-((2-formadoximinooxy)ethyl)-5-methyluridine, 5'-O-*tert*-Butyldiphenylsilyl-2'-O-(N,N dimethylaminooxyethyl)-5-methyluridine, 2'-O-(dimethylaminooxyethyl)-5-methyluridine, 5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine, 5'-O-DMT-2'-O-(2-N,N-dimethylaminooxyethyl)-5-methyluridine-3'-((2-cyanoethyl)-N,N-diisopropylphosphoramidite), 2'-(Aminooxyethoxy) nucleoside amidites, N2-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-((2-cyanoethyl)-N,N-diisopropylphosphoramidite), 2'-dimethylaminoethoxyethoxy (2'-DMAEOE) nucleoside amidites, 2'-O-(2(2-N,N-dimethylaminoethoxy)ethyl)-5-methyl uridine, 5'-O-dimethoxytrityl-2'-O-(2(2-N,N-dimethylaminoethoxy)-ethyl))-5-methyl uridine and 5'-O-Dimethoxytrityl-2'-O-

(2(2-N,N-dimethylaminoethoxy)-ethyl))-5-methyl uridine-3'-O-(cyanoethyl-N,N-diisopropyl)phosphoramidite.

Oligonucleotide and oligonucleoside synthesis:

Oligonucleotides: Unsubstituted and substituted phosphodiester (P=O) oligonucleotides are synthesized on an automated DNA synthesizer (Applied Biosystems model 394) using standard phosphoramidite chemistry with oxidation by iodine.

Phosphorothioates (P=S) are synthesized similar to phosphodiester oligonucleotides with the following exceptions: thiation was effected by utilizing a 10% w/v solution of 3,H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the oxidation of the phosphite linkages. The thiation reaction step time was increased to 180 sec and preceded by the normal capping step. After cleavage from the CPG column and deblocking in concentrated ammonium hydroxide at 55°C (12-16 hr), the oligonucleotides were recovered by precipitating with >3 volumes of ethanol from a 1 M NH_4OAc solution. Phosphinate oligonucleotides are prepared as described in U.S. Patent 5,508,270, herein incorporated by reference.

Alkyl phosphonate oligonucleotides are prepared as described in U.S. Patent 4,469,863, herein incorporated by reference.

3'-Deoxy-3'-methylene phosphonate oligonucleotides are prepared as described in U.S. Patents 5,610,289 or 5,625,050, herein incorporated by reference.

Phosphoramidite oligonucleotides are prepared as described in U.S. Patent, 5,256,775 or U.S. Patent 5,366,878, herein incorporated by reference.

Alkylphosphonothioate oligonucleotides are prepared as described in published PCT applications PCT/US94/00902 and PCT/US93/06976 (published as WO 94/17093 and WO 94/02499, respectively), herein incorporated by reference.

3'-Deoxy-3'-amino phosphoramidate oligonucleotides are prepared as described in U.S. Patent 5,476,925, herein incorporated by reference.

Phosphotriester oligonucleotides are prepared as described in U.S. Patent 5,023,243, herein incorporated by reference.

Borano phosphate oligonucleotides are prepared as described in U.S. Patents 5,130,302 and 5,177,198, both herein incorporated by reference.

Oligonucleosides: Methylenemethylimino linked oligonucleosides, also identified as MMI linked oligonucleosides, methylenedimethylhydrazo linked oligonucleosides, also identified as MDH linked oligonucleosides, and methylenecarbonylamino linked oligonucleosides, also identified as amide-3 linked oligonucleosides, and methyleneaminocarbonyl linked oligonucleosides, also identified as amide-4 linked

oligonucleosides, as well as mixed backbone oligomeric compounds having, for instance, alternating MMI and P=O or P=S linkages are prepared as described in U.S. Patents 5,378,825, 5,386,023, 5,489,677, 5,602,240 and 5,610,289, all of which are herein incorporated by reference.

Formacetal and thioformacetal linked oligonucleosides are prepared as described in U.S. Patents 5,264,562 and 5,264,564, herein incorporated by reference.

Ethylene oxide linked oligonucleosides are prepared as described in U.S. Patent 5,223,618, herein incorporated by reference.

RNA Synthesis:

In general, RNA synthesis chemistry is based on the selective incorporation of various protecting groups at strategic intermediary reactions. Although one of ordinary skill in the art will understand the use of protecting groups in organic synthesis, a useful class of protecting groups includes silyl ethers. In particular bulky silyl ethers are used to protect the 5'-hydroxyl in combination with an acid-labile orthoester protecting group on the 2'-hydroxyl. This set of protecting groups is then used with standard solid-phase synthesis technology. It is important to lastly remove the acid labile orthoester protecting group after all other synthetic steps. Moreover, the early use of the silyl protecting groups during synthesis ensures facile removal when desired, without undesired deprotection of 2' hydroxyl.

Following this procedure for the sequential protection of the 5'-hydroxyl in combination with protection of the 2'-hydroxyl by protecting groups that are differentially removed and are differentially chemically labile, RNA oligonucleotides were synthesized.

RNA oligonucleotides are synthesized in a stepwise fashion. Each nucleotide is added sequentially (3'- to 5'-direction) to a solid support-bound oligonucleotide. The first nucleoside at the 3'-end of the chain is covalently attached to a solid support. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator are added, coupling the second base onto the 5'-end of the first nucleoside. The support is washed and any unreacted 5'-hydroxyl groups are capped with acetic anhydride to yield 5'-acetyl moieties. The linkage is then oxidized to the more stable and ultimately desired P(V) linkage. At the end of the nucleotide addition cycle, the 5'-silyl group is cleaved with fluoride. The cycle is repeated for each subsequent nucleotide.

Following synthesis, the methyl protecting groups on the phosphates are cleaved in 30 minutes utilizing 1 M disodium-2-carbamoyl-2-cyanoethylene-1,1-dithiolate trihydrate (S_2Na_2) in DMF. The deprotection solution is washed from the solid support-bound oligonucleotide using water. The support is then treated with 40% methylamine in water for 10 minutes at 55°C. This

releases the RNA oligonucleotides into solution, deprotects the exocyclic amines, and modifies the 2'- groups. The oligonucleotides can be analyzed by anion exchange HPLC at this stage.

The 2'-orthoester groups are the last protecting groups to be removed. The ethylene glycol monoacetate orthoester protecting group developed by Dharmacon Research, Inc. (Lafayette, CO), is one example of a useful orthoester protecting group which, has the following important properties. It is stable to the conditions of nucleoside phosphoramidite synthesis and oligonucleotide synthesis. However, after oligonucleotide synthesis the oligonucleotide is treated with methylamine which not only cleaves the oligonucleotide from the solid support but also removes the acetyl groups from the orthoesters. The resulting 2-ethyl-hydroxyl substituents on the orthoester are less electron withdrawing than the acetylated precursor. As a result, the modified orthoester becomes more labile to acid-catalyzed hydrolysis. Specifically, the rate of cleavage is approximately 10 times faster after the acetyl groups are removed. Therefore, this orthoester possesses sufficient stability in order to be compatible with oligonucleotide synthesis and yet, when subsequently modified, permits deprotection to be carried out under relatively mild aqueous conditions compatible with the final RNA oligonucleotide product.

Additionally, methods of RNA synthesis are well known in the art (Scaringe, S. A. Ph.D. Thesis, University of Colorado, 1996; Scaringe, S. A., et al., *J. Am. Chem. Soc.*, **1998**, *120*, 11820-11821; Matteucci, M. D. and Caruthers, M. H. *J. Am. Chem. Soc.*, **1981**, *103*, 3185-3191; Beaucage, S. L. and Caruthers, M. H. *Tetrahedron Lett.*, **1981**, *22*, 1859-1862; Dahl, B. J., et al., *Acta Chem. Scand,*. **1990**, *44*, 639-641; Reddy, M. P., et al., *Tetrahedrom Lett.*, **1994**, *25*, 4311-4314; Wincott, F. et al., *Nucleic Acids Res.*, **1995**, *23*, 2677-2684; Griffin, B. E., et al., *Tetrahedron*, **1967**, *23*, 2301-2313; Griffin, B. E., et al., *Tetrahedron*, **1967**, *23*, 2315-2331).

The present invention is also useful for the preparation of oligomeric compounds incorporating at least one 2'-O-protected nucleoside. After incorporation and appropriate deprotection the 2'-O-protected nucleoside will be converted to a ribonucleoside at the position of incorporation. The number and position of the 2-ribonucleoside units in the final oligomeric compound can vary from one at any site or the strategy can be used to prepare up to a full 2'-OH modified oligomeric compound. All 2'-O-protecting groups amenable to the synthesis of oligomeric compounds are included in the present invention.

In general a protected nucleoside is attached to a solid support by for example a succinate linker. Then the oligonucleotide is elongated by repeated cycles of deprotecting the 5'-terminal hydroxyl group, coupling of a further nucleoside unit, capping and oxidation (alternatively sulfurization). In a more frequently used method of synthesis the completed

oligonucleotide is cleaved from the solid support with the removal of phosphate protecting groups and exocyclic amino protecting groups by treatment with an ammonia solution. Then a further deprotection step is normally required for the more specialized protecting groups used for the protection of 2'-hydroxyl groups which will give the fully deprotected oligonucleotide.

A large number of 2'-O-protecting groups have been used for the synthesis of oligoribonucleotides but over the years more effective groups have been discovered. The key to an effective 2'-O-protecting group is that it is capable of selectively being introduced at the 2'-O-position and that it can be removed easily after synthesis without the formation of unwanted side products. The protecting group also needs to be inert to the normal deprotecting, coupling, and capping steps required for oligoribonucleotide synthesis. Some of the protecting groups used initially for oligoribonucleotide synthesis included tetrahydropyran-1-yl and 4-methoxytetrahydropyran-4-yl. These two groups are not compatible with all 5'-O-protecting groups so modified versions were used with 5'-DMT groups such as 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp). Reese has identified a number of piperidine derivatives (like Fpmp) that are useful in the synthesis of oligoribonucleotides including 1-((chloro-4-methyl)phenyl)-4'-methoxypiperidin-4-yl (Reese *et al., Tetrahedron Lett.*, **1986**, *(27)*, 2291). Another approach was to replace the standard 5'-DMT (dimethoxytrityl) group with protecting groups that were removed under non-acidic conditions such as levulinyl and 9-fluorenylmethoxycarbonyl. Such groups enable the use of acid labile 2'-protecting groups for oligoribonucleotide synthesis. Another more widely used protecting group initially used for the synthesis of oligoribonucleotides was the *t*-butyldimethylsilyl group (Ogilvie *et al., Tetrahedron Lett.*, **1974**, 2861; Hakimelahi *et al., Tetrahedron Lett.*, **1981**, *(22)*, 2543; and Jones *et al., J. Chem. Soc. Perkin I.*, 2762). The 2'-O-protecting groups can require special reagents for their removal such as for example the *t*-butyldimethylsilyl group is normally removed after all other cleaving/deprotecting steps by treatment of the oligomeric compound with tetrabutylammonium fluoride (TBAF).

One group of researchers examined a number of 2'-O-protecting groups (Pitsch, S., *Chimia*, **2001**, *(55)*, 320-324.) The group examined fluoride labile and photolabile protecting groups that are removed using moderate conditions. One photolabile group that was examined was the (2-(nitrobenzyl)oxy)methyl (nbm) protecting group (Schwartz *et al., Bioorg. Med. Chem. Lett.*, **1992**, *(2)*, 1019.) Other groups examined included a number structurally related formaldehyde acetal-derived, 2'-O-protecting groups. Also prepared were a number of related protecting groups for preparing 2'-O-alkylated nucleoside phosphoramidites including 2'-O-((triisopropylsilyl)oxy)methyl (2'-O-CH_2-O-Si(iPr)$_3$, TOM). One 2'-O-protecting group that

was prepared to be used orthogonally to the TOM group was 2'-O-((*R*)-1-(2-nitrophenyl)ethyloxy)methyl) ((*R*)-mnbm).

Another strategy using a fluoride labile 5'-O-protecting group (non-acid labile) and an acid labile 2'-O-protecting group has been reported (Scaringe, Stephen A., *Methods*, **2001**, *(23)* 206-217). A number of possible silyl ethers were examined for 5'-O-protection and a number of acetals and orthoesters were examined for 2'-O-protection. The protection scheme that gave the best results was 5'-O-silyl ether-2'-ACE (5'-O-bis(trimethylsiloxy)cyclododecyloxysilyl ether (DOD)-2'-O-bis(2-acetoxyethoxy)methyl (ACE). This approach uses a modified phosphoramidite synthesis approach in that some different reagents are required that are not routinely used for RNA/DNA synthesis.

Although a lot of research has focused on the synthesis of oligoribonucleotides the main RNA synthesis strategies that are presently being used commercially include 5'-O-DMT-2'-O-*t*-butyldimethylsilyl (TBDMS), 5'-O-DMT-2'-O-(1(2-fluorophenyl)-4-methoxypiperidin-4-yl) (FPMP), 2'-O-((triisopropylsilyl)oxy)methyl (2'-O-CH_2-O-Si(iPr)$_3$ (TOM), and the 5'-O-silyl ether-2'-ACE (5'-O-bis(trimethylsiloxy)cyclododecyloxysilyl ether (DOD)-2'-O-bis(2-acetoxyethoxy)methyl (ACE). A current list of some of the major companies currently offering RNA products include Pierce Nucleic Acid Technologies, Dharmacon Research Inc., Ameri Biotechnologies Inc., and Integrated DNA Technologies, Inc. One company, Princeton Separations, is marketing an RNA synthesis activator advertised to reduce coupling times especially with TOM and TBDMS chemistries. Such an activator would also be amenable to the present invention.

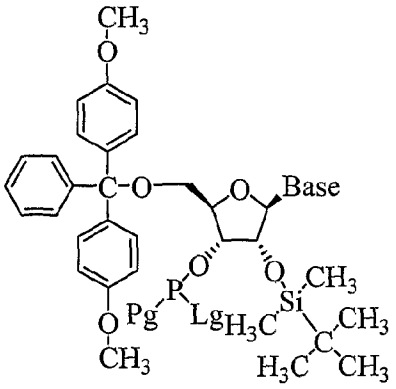
The structures corresponding to these protecting groups are shown below.

TBDMS = 5'-O-DMT-2'-O-*t*-butyldimethylsilyl;

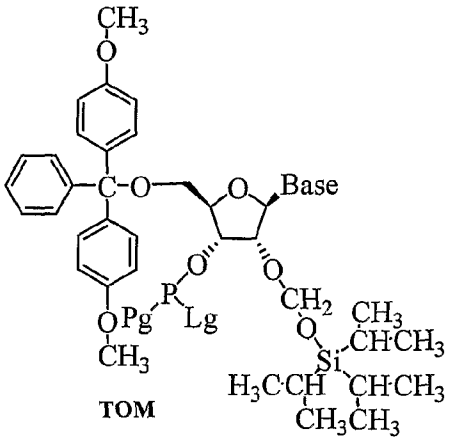
TOM = 2'-O-((triisopropylsilyl)oxy)methyl;

DOD/ACE = (5'-O-bis(trimethylsiloxy)cyclododecyloxysilyl ether-2'-O-bis(2-acetoxyethoxy)methyl

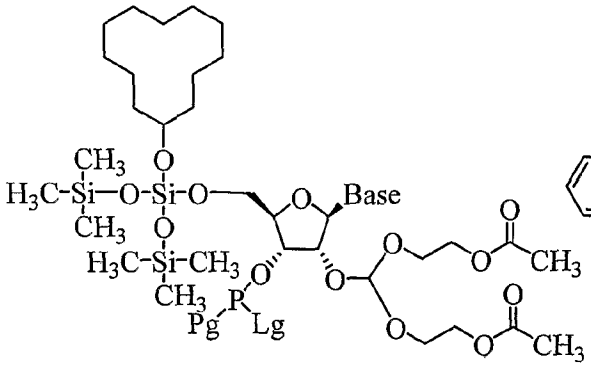
FPMP = 5'-O-DMT-2'-O-(1(2-fluorophenyl)-4-methoxypiperidin-4-yl)



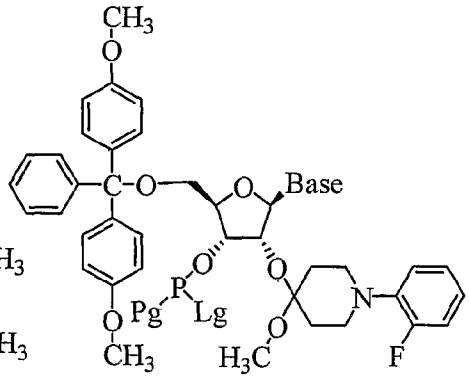
DMT/TBDMS



TOM



DOD/ACE



FPMP

All of the aforementioned RNA synthesis strategies are amenable to the present invention. Strategies that would be a hybrid of the above e.g. using a 5'-protecting group from one strategy with a 2'-O-protecting from another strategy is also amenable to the present invention.

The preparation of ribonucleotides and oligomeric compounds having at least one ribonucleoside incorporated and all the possible configurations falling in between these two extremes are encompassed by the present invention. The corresponding oligomeric compounds can be hybridized to further oligomeric compounds including oligoribonucleotides having regions of complementarity to form double-stranded (duplexed) oligomeric compounds.

The methods of preparing oligomeric compounds of the present invention can also be applied in the areas of drug discovery and target validation.

Oligonucleotide Isolation:

After cleavage from the controlled pore glass solid support and deblocking in concentrated ammonium hydroxide at 55°C for 12-16 hours, the oligonucleotides or oligonucleosides are recovered by precipitation out of 1 M NH_4OAc with >3 volumes of ethanol. Synthesized oligonucleotides were analyzed by electrospray mass spectroscopy (molecular

weight determination) and by capillary gel electrophoresis and judged to be at least 70% full length material. The relative amounts of phosphorothioate and phosphodiester linkages obtained in the synthesis was determined by the ratio of correct molecular weight relative to the –16 amu product (+/-32 +/-48). For some studies oligonucleotides were purified by HPLC, as described by Chiang *et al., J. Biol. Chem.* **1991**, *266*, 18162-18171. Results obtained with HPLC-purified material were similar to those obtained with non-HPLC purified material.

Oligonucleotide Synthesis - 96 Well Plate Format:

Oligonucleotides were synthesized via solid phase P(III) phosphoramidite chemistry on an automated synthesizer capable of assembling 96 sequences simultaneously in a 96-well format. Phosphodiester internucleotide linkages were afforded by oxidation with aqueous iodine. Phosphorothioate internucleotide linkages were generated by sulfurization utilizing 3,H-1,2 benzodithiole-3-one 1,1 dioxide (Beaucage Reagent) in anhydrous acetonitrile. Standard base-protected beta-cyanoethyl-diiso-propyl phosphoramidites were purchased from commercial vendors (e.g. PE-Applied Biosystems, Foster City, CA, or Pharmacia, Piscataway, NJ). Non-standard nucleosides are synthesized as per standard or patented methods. They are utilized as base protected beta-cyanoethyldiisopropyl phosphoramidites.

Oligonucleotides were cleaved from support and deprotected with concentrated NH_4OH at elevated temperature (55-60°C) for 12-16 hours and the released product then dried *in vacuo*. The dried product was then re-suspended in sterile water to afford a master plate from which all analytical and test plate samples are then diluted utilizing robotic pipettors.

Oligonucleotide Analysis – 96-Well Plate Format:

The concentration of oligonucleotide in each well was assessed by dilution of samples and UV absorption spectroscopy. The full-length integrity of the individual products was evaluated by capillary electrophoresis (CE) in either the 96-well format (Beckman P/ACE™ MDQ) or, for individually prepared samples, on a commercial CE apparatus (e.g., Beckman P/ACE™ 5000, ABI 270). Base and backbone composition was confirmed by mass analysis of the oligomeric compounds utilizing electrospray-mass spectroscopy. All assay test plates were diluted from the master plate using single and multi-channel robotic pipettors. Plates were judged to be acceptable if at least 85% of the oligomeric compounds on the plate were at least 85% full length.

For double-stranded compounds of the invention, once synthesized, the complementary strands are annealed. The single strands are aliquoted and diluted to a concentration of 50 μM. Once diluted, 30 μL of each strand is combined with 15 μL of a 5X solution of annealing buffer. The final concentration of the buffer is 100 mM potassium acetate, 30 mM HEPES-KOH pH 7.4,

and 2mM magnesium acetate. The final volume is 75 µL. This solution is incubated for 1 minute at 90°C and then centrifuged for 15 seconds. The tube is allowed to sit for 1 hour at 37°C at which time the double-stranded compounds are used in experimentation. The final concentration of the duplexed compound is 20 µM. This solution can be stored frozen (-20°C) and freeze-thawed up to 5 times.

Once prepared, the double-stranded compounds are evaluated for their ability to modulate target levels, expression or function. When cells reach 80% confluency, they are treated with synthetic double-stranded compounds comprising at least one oligomeric compound of the invention. For cells grown in 96-well plates, wells are washed once with 200 µL OPTI-MEM™-1 reduced-serum medium (Gibco BRL) and then treated with 130 µL of OPTI-MEM™-1 containing 12 µg/mL LIPOFECTIN™ (Invitrogen Corporation, Carlsbad, CA) and the desired double stranded compound at a final concentration of 200 nM. After 5 hours of treatment, the medium is replaced with fresh medium. Cells are harvested 16 hours after treatment, at which time RNA is isolated and target reduction measured by real-time RT-PCR.

Specific examples of oligomeric compounds useful in this invention include oligonucleotides containing modified e.g. non-naturally occurring internucleoside linkages. As defined in this specification, oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom and internucleoside linkages that do not have a phosphorus atom. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

In the *C. elegans* system, modification of the internucleotide linkage (phosphorothioate) did not significantly interfere with RNAi activity. Based on this observation, it is suggested that certain oligomeric compounds of the invention can also have one or more modified internucleoside linkages. A suitable phosphorus-containing modified internucleoside linkage is the phosphorothioate internucleoside linkage.

Modified oligonucleotide backbones (internucleoside linkages) containing a phosphorus atom therein include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more

internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage. Oligonucleotides having inverted polarity comprise a single 3' to 3' linkage at the 3'-most internucleotide linkage i.e. a single inverted nucleoside residue which may be abasic (the nucleobase is missing or has a hydroxyl group in place thereof). Various salts, mixed salts and free acid forms are also included.

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S.: 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,194,599; 5,565,555; 5,527,899; 5,721,218; 5,672,697 and 5,625,050, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

In other embodiments of the invention, oligomeric compounds have one or more phosphorothioate and/or heteroatom internucleoside linkages, in particular $-CH_2-NH-O-CH_2-$, $-CH_2-N(CH_3)-O-CH_2-$ (known as a methylene (methylimino) or MMI backbone), $-CH_2-O-N(CH_3)-CH_2-$, $-CH_2-N(CH_3)-N(CH_3)-CH_2-$ and $-O-N(CH_3)-CH_2-CH_2-$ (wherein the native phosphodiester internucleotide linkage is represented as $-O-P(=O)(OH)-O-CH_2-$). The MMI type internucleoside linkages are disclosed in the above referenced U.S. patent 5,489,677. Amide internucleoside linkages are disclosed in the above referenced U.S. patent 5,602,240.

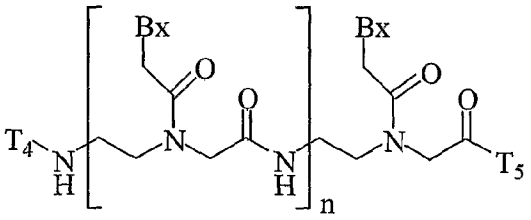
Modified oligonucleotide backbones (internucleoside linkages) that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; riboacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S.: 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; 5,792,608; 5,646,269 and 5,677,439,

certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

Another group of oligomeric compounds amenable to the present invention includes oligonucleotide mimetics. The term mimetic as it is applied to oligonucleotides is intended to include oligomeric compounds wherein only the furanose ring or both the furanose ring and the internucleotide linkage are replaced with novel groups, replacement of only the furanose ring is also referred to in the art as being a sugar surrogate. The heterocyclic base moiety or a modified heterocyclic base moiety is maintained for hybridization with an appropriate target nucleic acid. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA oligomeric compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA oligomeric compounds include, but are not limited to, U.S.: 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Teaching of PNA oligomeric compounds can be found in Nielsen *et al.*, *Science*, **1991**, 254, 1497-1500.

PNA has been modified to incorporate numerous modifications since the basic PNA structure was first prepared. The basic structure is shown below:



wherein

Bx is a heterocyclic base moiety;

T_4 is hydrogen, an amino protecting group, $-C(O)R_5$, substituted or unsubstituted C_1-C_{10} alkyl, substituted or unsubstituted C_2-C_{10} alkenyl, substituted or unsubstituted C_2-C_{10} alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group, a reporter group, a conjugate group, a D or L α-amino acid linked via the α-carboxyl group or optionally through the ω-carboxyl group when the amino acid is aspartic acid or glutamic acid or a peptide derived from D, L or mixed D and L amino acids linked through a carboxyl group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

T_5 is -OH, $-N(Z_1)Z_2$, R_5, D or L α-amino acid linked via the α-amino group or optionally through the ω-amino group when the amino acid is lysine or ornithine or a peptide derived from D, L or mixed D and L amino acids linked through an amino group, a chemical functional group, a reporter group or a conjugate group;

Z_1 is hydrogen, C_1-C_6 alkyl, or an amino protecting group;

Z_2 is hydrogen, C_1-C_6 alkyl, an amino protecting group, $-C(=O)-(CH_2)_n-J-Z_3$, a D or L α-amino acid linked via the α-carboxyl group or optionally through the ω-carboxyl group when the amino acid is aspartic acid or glutamic acid or a peptide derived from D, L or mixed D and L amino acids linked through a carboxyl group;

Z_3 is hydrogen, an amino protecting group, $-C_1$-C_6 alkyl, $-C(=O)-CH_3$, benzyl, benzoyl, or $-(CH_2)_n-N(H)Z_1$;

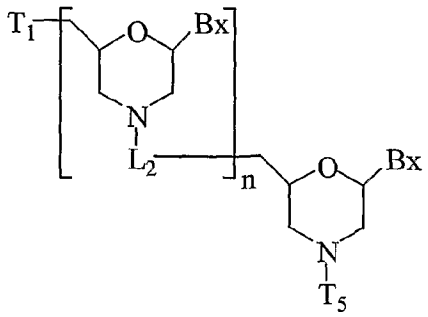
each J is O, S or NH;

R_5 is a carbonyl protecting group; and

n is from 2 to about 450.

Another class of oligonucleotide mimetic that has been studied is based on linked morpholino units (morpholino nucleic acid) having heterocyclic bases attached to the morpholino ring. A number of linking groups have been reported that link the morpholino monomeric units in a morpholino nucleic acid. A suitable class of linking groups have been selected to give a non-ionic oligomeric compound. The non-ionic morpholino-based oligomeric compounds are less likely to have undesired interactions with cellular proteins. Morpholino-based oligomeric compounds are non-ionic mimics of oligonucleotides which are less likely to form undesired interactions with cellular proteins (Dwaine A. Braasch and David R. Corey, Biochemistry, 2002, 41(14), 4503-4510). Morpholino-based oligomeric compounds are disclosed in U.S. Patent 5,034,506, issued July 23, 1991. The morpholino class of oligomeric compounds have been prepared having a variety of different linking groups joining the monomeric subunits.

Morpholino nucleic acids have been prepared having a variety of different linking groups (L_2) joining the monomeric subunits. The basic formula is shown below:



wherein

T_1 is hydroxyl or a protected hydroxyl;

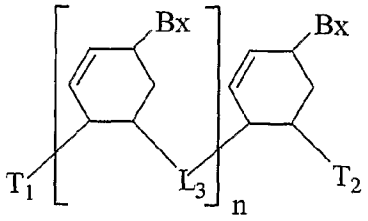
T_5 is hydrogen or a phosphate or phosphate derivative;

L_2 is a linking group; and

n is from 2 to about 450.

Another class of oligonucleotide mimetic is referred to as cyclohexenyl nucleic acids (CeNA). The furanose ring normally present in an DNA/RNA molecule is replaced with a cyclohenyl ring. CeNA DMT protected phosphoramidite monomers have been prepared and used for oligomeric compound synthesis following classical phosphoramidite chemistry. Fully modified CeNA oligomeric compounds and oligonucleotides having specific positions modified with CeNA have been prepared and studied (see Wang et al., J. Am. Chem. Soc., 2000, 122, 8595-8602). In general the incorporation of CeNA monomers into a DNA chain increases its stability of a DNA/RNA hybrid. CeNA oligoadenylates formed complexes with RNA and DNA complements with similar stability to the native complexes. The study of incorporating CeNA structures into natural nucleic acid structures was shown by NMR and circular dichroism to proceed with easy conformational adaptation. Furthermore the incorporation of CeNA into a sequence targeting RNA was stable to serum and able to activate *E. coli* RNase resulting in cleavage of the target RNA strand.

The general formula of CeNA is shown below:



wherein

each Bx is a heterocyclic base moiety;

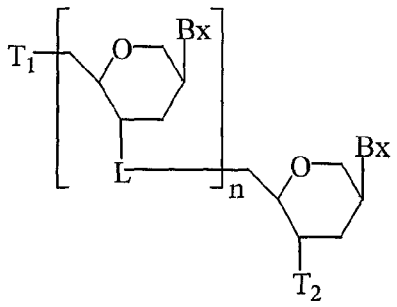
T_1 is hydroxyl or a protected hydroxyl;

T_2 is hydroxyl or a protected hydroxyl;

L_3 is a linking group; and

n is from 2 to about 450.

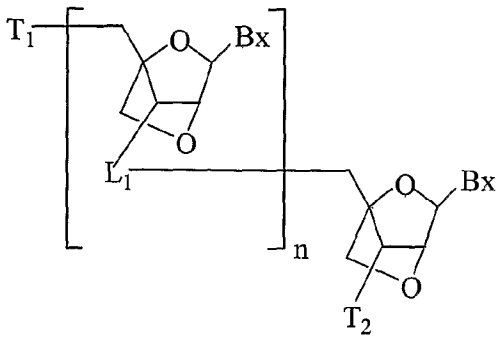
Another class of oligonucleotide mimetic (anhydrohexitol nucleic acid) can be prepared from one or more anhydrohexitol nucleosides (see, Wouters and Herdewijn, Bioorg. Med. Chem. Lett., 1999, 9, 1563-1566) and would have the general formula:



Another group of modifications includes nucleosides having sugar moieties that are bicyclic thereby locking the sugar conformational geometry. The most studied of these nucleosides is a bicyclic sugar moiety having a 4'-CH_2-O-2' bridge. As can be seen in the structure below the 2'-O- has been linked via a methylene group to the 4' carbon. This bridge attaches under the sugar as shown forcing the sugar ring into a locked 3'-endo conformation geometry. The ∀-L nucleoside has also been reported wherein the linkage is above the ring and the heterocyclic base is in the ∀ rather than the ∃-conformation (see U.S. Patent Application Publication No.: Application 2003/0087230). The xylo analog has also been prepared (see U.S. Patent Application Publication No.: 2003/0082807). The preferred bridge for a locked nucleic acid (LNA) is 4'-(-CH_2-)$_n$-O-2' wherein n is 1 or 2. The literature is confusing when the term locked nucleic acid is used but in general locked nucleic acids refers to n=1, ENA™ refers to n=2 (Kaneko et al., U.S. Patent Application Publication No.: US 2002/0147332, Singh et al., Chem. Commun., 1998, 4, 455-456, also see U.S. Patents 6,268,490 and 6,670,461 and U.S. Patent Application Publication No.: US 2003/0207841). However the term locked nucleic acids can also be used in a more general sense to describe any bicyclic sugar moiety that has a locked conformation.

ENA™ along with LNA (n=1) have been studied more than the myriad of other analogs. Oligomeric comounds incorporating LNA and ENA analogs display very high duplex thermal stabilities with complementary DNA and RNA (Tm = +3 to +10 C), stability towards 3'-exonucleolytic degradation and good solubility properties.

The basic structure of LNA showing the bicyclic ring system is shown below:



wherein

each Bx is a heterocyclic base moiety;

each L_1 is an internucleoside linking group;

T_1 is hydroxyl or a protected hydroxyl;

T_2 is hydroxyl or a protected hydroxyl, and

n is from 1 to about 80.

The conformations of LNAs determined by 2D NMR spectroscopy have shown that the locked orientation of the LNA nucleotides, both in single-stranded LNA and in duplexes, constrains the phosphate backbone in such a way as to introduce a higher population of the N-type conformation (Petersen et al., J. Mol. Recognit., 2000, 13, 44-53). These conformations are associated with improved stacking of the nucleobases (Wengel et al., Nucleosides Nucleotides, 1999, 18, 1365-1370).

LNA has been shown to form exceedingly stable LNA:LNA duplexes (Koshkin et al., J. Am. Chem. Soc., 1998, 120, 13252-13253). LNA:LNA hybridization was shown to be the most thermally stable nucleic acid type duplex system, and the RNA-mimicking character of LNA was established at the duplex level. Introduction of 3 LNA monomers (T or A) significantly increased melting points (Tm = +15/+11) toward DNA complements. The universality of LNA-mediated hybridization has been stressed by the formation of exceedingly stable LNA:LNA duplexes. The RNA-mimicking of LNA was reflected with regard to the N-type conformational restriction of the monomers and to the secondary structure of the LNA:RNA duplex.

LNAs also form duplexes with complementary DNA, RNA or LNA with high thermal affinities. Circular dichroism (CD) spectra show that duplexes involving fully modified LNA (esp. LNA:RNA) structurally resemble an A-form RNA:RNA duplex. Nuclear magnetic resonance (NMR) examination of an LNA:DNA duplex confirmed the 3'-endo conformation of an LNA monomer. Recognition of double-stranded DNA has also been demonstrated suggesting strand invasion by LNA. Studies of mismatched sequences show that LNAs obey the Watson-

Crick base pairing rules with generally improved selectivity compared to the corresponding unmodified reference strands.

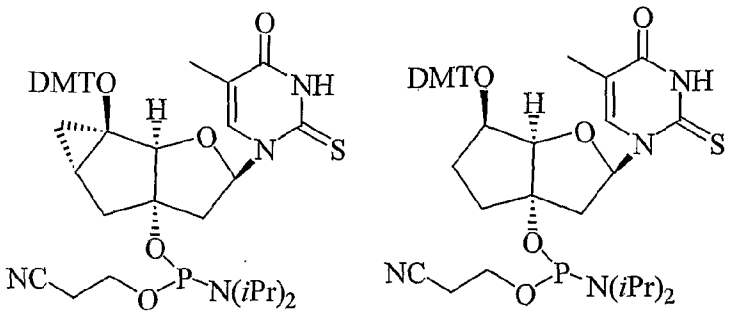
Novel types of LNA-oligomeric compounds, as well as the LNAs, are useful in a wide range of diagnostic and therapeutic applications. Among these are antisense applications, PCR applications, strand-displacement oligomers, substrates for nucleic acid polymerases and generally as nucleotide based drugs.

Potent and nontoxic antisense oligonucleotides containing LNAs have been described (Wahlestedt et al., Proc. Natl. Acad. Sci. U. S. A., 2000, 97, 5633-5638.) The authors have demonstrated that LNAs confer several desired properties to antisense agents. LNA/DNA copolymers were not degraded readily in blood serum and cell extracts. LNA/DNA copolymers exhibited potent antisense activity in assay systems as disparate as G-protein-coupled receptor signaling in living rat brain and detection of reporter genes in *Escherichia coli*. LIPOFECTIN™ -mediated efficient delivery of LNA into living human breast cancer cells has also been accomplished.

The synthesis and preparation of the LNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (Koshkin et al., Tetrahedron, 1998, 54, 3607-3630). LNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

The first analogs of LNA, phosphorothioate-LNA and 2'-thio-LNAs, have also been prepared (Kumar et al., Bioorg. Med. Chem. Lett., 1998, 8, 2219-2222). Preparation of locked nucleoside analogs containing oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (Wengel et al., PCT International Application WO 98-DK393 19980914). Furthermore, synthesis of 2'-amino-LNA, a novel conformationally restricted high-affinity oligonucleotide analog with a handle has been described in the art (Singh et al., J. Org. Chem., 1998, 63, 10035-10039). In addition, 2'-Amino- and 2'-methylamino-LNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

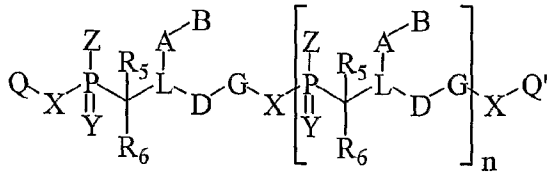
Some oligonucleotide mimetics have been prepared to incude bicyclic and tricyclic nucleoside analogs having the formulas (amidite monomers shown):



(see Steffens et al., Helv. Chim. Acta, 1997, 80, 2426-2439; Steffens et al., J. Am. Chem. Soc., 1999, 121, 3249-3255; and Renneberg et al., J. Am. Chem. Soc., 2002, 124, 5993-6002). These modified nucleoside analogs have been oligomerized using the phosphoramidite approach and the resulting oligomeric compounds containing tricyclic nucleoside analogs have shown increased thermal stabilities (Tm's) when hybridized to DNA, RNA and itself. Oligomeric compounds containing bicyclic nucleoside analogs have shown thermal stabilities approaching that of DNA duplexes.

Another class of oligonucleotide mimetic is referred to as phosphonomonoester nucleic acid and incorporates a phosphorus group in the backbone. This class of olignucleotide mimetic is reported to have useful physical and biological and pharmacological properties in the areas of inhibiting gene expression (antisense oligonucleotides, ribozymes, sense oligonucleotides and triplex-forming oligonucleotides), as probes for the detection of nucleic acids and as auxiliaries for use in molecular biology.

The general formula (for definitions of Markush variables see: U.S. Patents 5,874,553 and 6,127,346 herein incorporated by reference in their entirety) is shown below.



Another oligonucleotide mimetic has been reported wherein the furanosyl ring has been replaced by a cyclobutyl moiety.

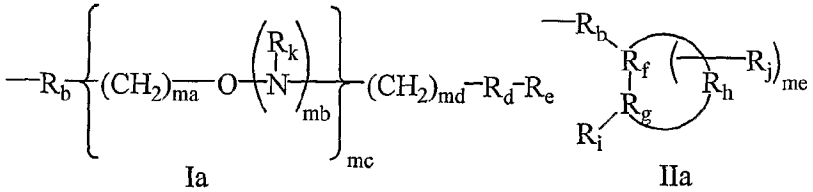
Modified sugars

Oligomeric compounds of the invention may also contain one or more substituted sugar moieties. These oligomeric compounds comprise a sugar substituent group selected from: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly suitable are $O((CH_2)_nO)_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$,

$O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON((CH_2)_nCH_3)_2$, where n and m are from 1 to about 10. Some oligonucleotides comprise a sugar substituent group selected from: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. One modification includes 2'-methoxyethoxy (2'-O-$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., Helv. Chim. Acta, 1995, 78, 486-504) i.e., an alkoxyalkoxy group. One modification includes 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples hereinbelow, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethyl-amino-ethoxy-ethyl or 2'-DMAEOE), i.e., 2'-O-CH_2-O-CH_2-$N(CH_3)_2$.

Other sugar substituent groups include methoxy (-O-CH_3), aminopropoxy (-$OCH_2CH_2CH_2NH_2$), allyl (-CH_2-CH=CH_2), -O-allyl (-O-CH_2-CH=CH_2) and fluoro (F). 2'-Sugar substituent groups may be in the arabino (up) position or ribo (down) position. One 2'-arabino modification is 2'-F. Similar modifications may also be made at other positions on the oligomeric compound, particularly the 3' position of the sugar on the 3' terminal nucleoside or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligomeric compounds may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,792,747; and 5,700,920, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

Representative sugar substituent groups include groups of formula I_a or II_a:



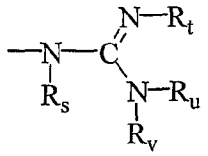
Ia IIa

wherein:

R_b is O, S or NH;

R_d is a single bond, O, S or C(=O);

R_e is C_1-C_{10} alkyl, $N(R_k)(R_m)$, $N(R_k)(R_n)$, $N{=}C(R_p)(R_q)$, $N{=}C(R_p)(R_r)$ or has formula III_a;



IIIa

R_p and R_q are each independently hydrogen or C_1-C_{10} alkyl;

R_r is $-R_x$-R_y;

each R_s, R_t, R_u and R_v is, independently, hydrogen, $C(O)R_w$, substituted or unsubstituted C_1-C_{10} alkyl, substituted or unsubstituted C_2-C_{10} alkenyl, substituted or unsubstituted C_2-C_{10} alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

or optionally, R_u and R_v, together form a phthalimido moiety with the nitrogen atom to which they are attached;

each R_w is, independently, substituted or unsubstituted C_1-C_{10} alkyl, trifluoromethyl, cyanoethyloxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or aryl;

R_k is hydrogen, a nitrogen protecting group or $-R_x$-R_y;

R_p is hydrogen, a nitrogen protecting group or $-R_x$-R_y;

R_x is a bond or a linking moiety;

R_y is a chemical functional group, a conjugate group or a solid support medium;

each R_m and R_n is, independently, H, a nitrogen protecting group, substituted or unsubstituted C_1-C_{10} alkyl, substituted or unsubstituted C_2-C_{10} alkenyl, substituted or unsubstituted C_2-C_{10} alkynyl, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl, alkynyl; NH_3^+, $N(R_u)(R_v)$, guanidino and acyl where said acyl is an acid amide or an ester;

or R_m and R_n, together, are a nitrogen protecting group, are joined in a ring structure that optionally includes an additional heteroatom selected from N and O or are a chemical functional group;

R_i is OR_z, SR_z, or $N(R_z)_2$;

each R_z is, independently, H, C_1-C_8 alkyl, C_1-C_8 haloalkyl, $C(=NH)N(H)R_u$, $C(=O)N(H)R_u$ or $OC(=O)N(H)R_u$;

R_f, R_g and R_h comprise a ring system having from about 4 to about 7 carbon atoms or having from about 3 to about 6 carbon atoms and 1 or 2 heteroatoms wherein said heteroatoms are selected from oxygen, nitrogen and sulfur and wherein said ring system is aliphatic, unsaturated aliphatic, aromatic, or saturated or unsaturated heterocyclic;

R_j is alkyl or haloalkyl having 1 to about 10 carbon atoms, alkenyl having 2 to about 10 carbon atoms, alkynyl having 2 to about 10 carbon atoms, aryl having 6 to about 14 carbon atoms, $N(R_k)(R_m)$ OR_k, halo, SR_k or CN;

m_a is 1 to about 10;

each mb is, independently, 0 or 1;

mc is 0 or an integer from 1 to 10;

md is an integer from 1 to 10;

me is from 0, 1 or 2; and

provided that when mc is 0, md is greater than 1.

Representative substituents groups are disclosed in U.S. Patent Application Serial No. 09/130,973, filed August 7, 1998, entitled "Capped 2'-Oxyethoxy Oligonucleotides," hereby incorporated by reference in its entirety.

Representative cyclic substituent groups are disclosed in U.S. Patent Application Serial No. 09/123,108, filed July 27, 1998, entitled "RNA Targeted 2'-Oligomeric compounds that are Conformationally Preorganized," hereby incorporated by reference in its entirety.

Particular sugar substituent groups include $O((CH_2)_nO)_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON((CH_2)_nCH_3))_2$, where n and m are from 1 to about 10.

Representative guanidino substituent groups are disclosed in U.S. Patent Application 09/349,040, entitled "Functionalized Oligomers," filed July 7, 1999, hereby incorporated by reference in its entirety.

Representative acetamido substituent groups are disclosed in U.S. Patent 6,147,200 which is hereby incorporated by reference in its entirety.

Representative dimethylaminoethyloxyethyl substituent groups are disclosed in International Patent Application PCT/US99/17895, entitled "2'-O-Dimethylaminoethyloxyethyl-Oligomeric compounds", filed August 6, 1999, hereby incorporated by reference in its entirety.

Synthesis of Chimeric Oligonucleotides:

Chimeric oligonucleotides, oligonucleosides or mixed oligonucleotides/oligonucleosides of the invention can be of several different types. These include a first type wherein the "gap" segment of linked nucleosides is positioned between 5' and

3' "wing" segments of linked nucleosides and a second "open end" type wherein the "gap" segment is located at either the 3' or the 5' terminus of the oligomeric compound. Oligonucleotides of the first type are also known in the art as "gapmers" or gapped oligonucleotides. Oligonucleotides of the second type are also known in the art as "hemimers" or "wingmers."

(2'-O-Me)—(2'-deoxy)—(2'-O-Me) Chimeric Phosphorothioate Oligonucleotides

Chimeric oligonucleotides having 2'-O-alkyl phosphorothioate and 2'-deoxy phosphorothioate oligonucleotide segments are synthesized using an Applied Biosystems automated DNA synthesizer Model 394, as above. Oligonucleotides are synthesized using the automated synthesizer and 2'-deoxy-5'-dimethoxytrityl-3'-O-phosphoramidite for the DNA portion and 5'-dimethoxytrityl-2'-O-methyl-3'-O-phosphoramidite for 5' and 3' wings. The standard synthesis cycle is modified by incorporating coupling steps with increased reaction times for the 5'-dimethoxytrityl-2'-O-methyl-3'-O-phosphoramidite. The fully protected oligonucleotide is cleaved from the support and deprotected in concentrated ammonia (NH_4OH) for 12-16 hr at 55°C. The deprotected oligo is then recovered by an appropriate method (precipitation, column chromatography, volume reduced *in vacuo* and analyzed spetrophotometrically for yield and for purity by capillary electrophoresis and by mass spectrometry.

(2'-O-(2-Methoxyethyl))—(2'-deoxy)—(2'-O-(Methoxyethyl)) Chimeric Phosphorothioate Oligonucleotides

(2'-O-(2-methoxyethyl))—(2'-deoxy)—(-2'-O-(methoxyethyl)) chimeric phosphorothioate oligonucleotides were prepared as per the procedure above for the 2'-O-methyl chimeric oligonucleotide, with the substitution of 2'-O-(methoxyethyl) amidites for the 2'-O-methyl amidites.

(2'-O-(2-Methoxyethyl)Phosphodiester)—(2'-deoxy Phosphorothioate)—(2'-O-(2-Methoxyethyl) Phosphodiester) Chimeric Oligonucleotides

(2'-O-(2-methoxyethyl phosphodiester)--(2'-deoxy phosphorothioate)--(2'-O-(methoxyethyl) phosphodiester) chimeric oligonucleotides are prepared as per the above procedure for the 2'-O-methyl chimeric oligonucleotide with the substitution of 2'-O-(methoxyethyl) amidites for the 2'-O-methyl amidites, oxidation with iodine to generate the phosphodiester internucleotide linkages within the wing portions of the chimeric structures and sulfurization utilizing 3,H-1,2 benzodithiole-3-one 1,1 dioxide (Beaucage Reagent) to generate the phosphorothioate internucleotide linkages for the center gap.

Other chimeric oligonucleotides, chimeric oligonucleosides and mixed chimeric oligonucleotides/oligonucleosides are synthesized according to U.S. Patent 5,623,065, herein incorporated by reference.

The terms used to describe the conformational geometry of homoduplex nucleic acids are "A Form" for RNA and "B Form" for DNA. The respective conformational geometry for RNA and DNA duplexes was determined from X-ray diffraction analysis of nucleic acid fibers (Arnott and Hukins, Biochem. Biophys. Res. Comm., 1970, 47, 1504.) In general, RNA:RNA duplexes are more stable and have higher melting temperatures (Tm's) than DNA:DNA duplexes (Sanger et al., Principles of Nucleic Acid Structure, 1984, Springer-Verlag; New York, NY.; Lesnik et al., Biochemistry, 1995, 34, 10807-10815; Conte et al., Nucleic Acids Res., 1997, 25, 2627-2634). The increased stability of RNA has been attributed to several structural features, most notably the improved base stacking interactions that result from an A-form geometry (Searle et al., Nucleic Acids Res., 1993, 21, 2051-2056). The presence of the 2' hydroxyl in RNA biases the sugar toward a C3' endo pucker, i.e., also designated as Northern pucker, which causes the duplex to favor the A-form geometry. In addition, the 2' hydroxyl groups of RNA can form a network of water mediated hydrogen bonds that help stabilize the RNA duplex (Egli et al., Biochemistry, 1996, 35, 8489-8494). On the other hand, deoxy nucleic acids prefer a C2' endo sugar pucker, i.e., also known as Southern pucker, which is thought to impart a less stable B-form geometry (Sanger, W. (1984) Principles of Nucleic Acid Structure, Springer-Verlag, New York, NY). As used herein, B-form geometry is inclusive of both C2'-endo pucker and O4'-endo pucker. This is consistent with Berger, et. al., Nucleic Acids Research, 1998, 26, 2473-2480, who pointed out that in considering the furanose conformations which give rise to B-form duplexes consideration should also be given to a O4'-endo pucker contribution.

DNA:RNA hybrid duplexes, however, are usually less stable than pure RNA:RNA duplexes, and depending on their sequence may be either more or less stable than DNA:DNA duplexes (Searle et al., Nucleic Acids Res., 1993, 21, 2051-2056). The structure of a hybrid duplex is intermediate between A- and B-form geometries, which may result in poor stacking interactions (Lane et al., Eur. J. Biochem., 1993, 215, 297-306; Fedoroff et al., J. Mol. Biol., 1993, 233, 509-523; Gonzalez et al., Biochemistry, 1995, 34, 4969-4982; Horton et al., J. Mol. Biol., 1996, 264, 521-533). The stability of the duplex formed between a target RNA and a synthetic sequence is central to therapies such as, but not limited to, antisense mechanisms, including RNase H-mediated and RNA interference mechanisms, as these mechanisms involved the hybridization of a synthetic sequence strand to an RNA target strand. In the case of RNase

H, effective inhibition of the mRNA requires that the antisense sequence achieve at least a threshold of hybridization.

One routinely used method of modifying the sugar puckering is the substitution of the sugar at the 2'-position with a substituent group that influences the sugar geometry. The influence on ring conformation is dependent on the nature of the substituent at the 2'-position. A number of different substituents have been studied to determine their sugar puckering effect. For example, 2'-halogens have been studied showing that the 2'-fluoro derivative exhibits the largest population (65%) of the C3'-endo form, and the 2'-iodo exhibits the lowest population (7%). The populations of adenosine (2'-OH) versus deoxyadenosine (2'-H) are 36% and 19%, respectively. Furthermore, the effect of the 2'-fluoro group of adenosine dimers (2'-deoxy-2'-fluoroadenosine - 2'-deoxy-2'-fluoro-adenosine) is also correlated to the stabilization of the stacked conformation.

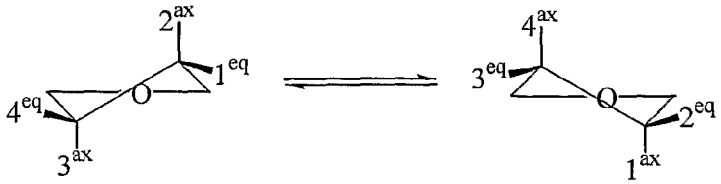
As expected, the relative duplex stability can be enhanced by replacement of 2'-OH groups with 2'-F groups thereby increasing the C3'-endo population. It is assumed that the highly polar nature of the 2'-F bond and the extreme preference for C3'-endo puckering may stabilize the stacked conformation in an A-form duplex. Data from UV hypochromicity, circular dichroism, and ^{1}H NMR also indicate that the degree of stacking decreases as the electronegativity of the halo substituent decreases. Furthermore, steric bulk at the 2'-position of the sugar moiety is better accommodated in an A-form duplex than a B-form duplex. Thus, a 2'-substituent on the 3'-terminus of a dinucleoside monophosphate is thought to exert a number of effects on the stacking conformation: steric repulsion, furanose puckering preference, electrostatic repulsion, hydrophobic attraction, and hydrogen bonding capabilities. These substituent effects are thought to be determined by the molecular size, electronegativity, and hydrophobicity of the substituent. Melting temperatures of complementary strands is also increased with the 2'-substituted adenosine diphosphates. It is not clear whether the 3'-endo preference of the conformation or the presence of the substituent is responsible for the increased binding. However, greater overlap of adjacent bases (stacking) can be achieved with the 3'-endo conformation.

Nucleoside conformation is influenced by various factors including substitution at the 2', 3' or 4'-positions of the pentofuranosyl sugar. Electronegative substituents generally prefer the axial positions, while sterically demanding substituents generally prefer the equatorial positions (Principles of Nucleic Acid Structure, Wolfgang Sanger, 1984, Springer-Verlag.) Modification of the 2' position to favor the 3'-endo conformation can be achieved while maintaining the 2'-OH as a recognition element, as illustrated in Figure 2, below (Gallo et al., Tetrahedron (2001), 57, 5707-5713. Harry-O'kuru et al., J. Org. Chem., (1997), 62(6), 1754-

1759 and Tang et al., J. Org. Chem. (1999), 64, 747-754.) Alternatively, preference for the 3'-endo conformation can be achieved by deletion of the 2'-OH as exemplified by 2'deoxy-2'F-nucleosides (Kawasaki et al., J. Med. Chem. (1993), 36, 831-841), which adopts the 3'-endo conformation positioning the electronegative fluorine atom in the axial position. Other modifications of the ribose ring, for example substitution at the 4'-position to give 4'-F modified nucleosides (Guillerm et al., Bioorganic and Medicinal Chemistry Letters (1995), 5, 1455-1460 and Owen et al., J. Org. Chem. (1976), 41, 3010-3017), or for example modification to yield methanocarba nucleoside analogs (Jacobson et al., J. Med. Chem. Lett. (2000), 43, 2196-2203 and Lee et al., Bioorganic and Medicinal Chemistry Letters (2001), 11, 1333-1337) also induce preference for the 3'-endo conformation.

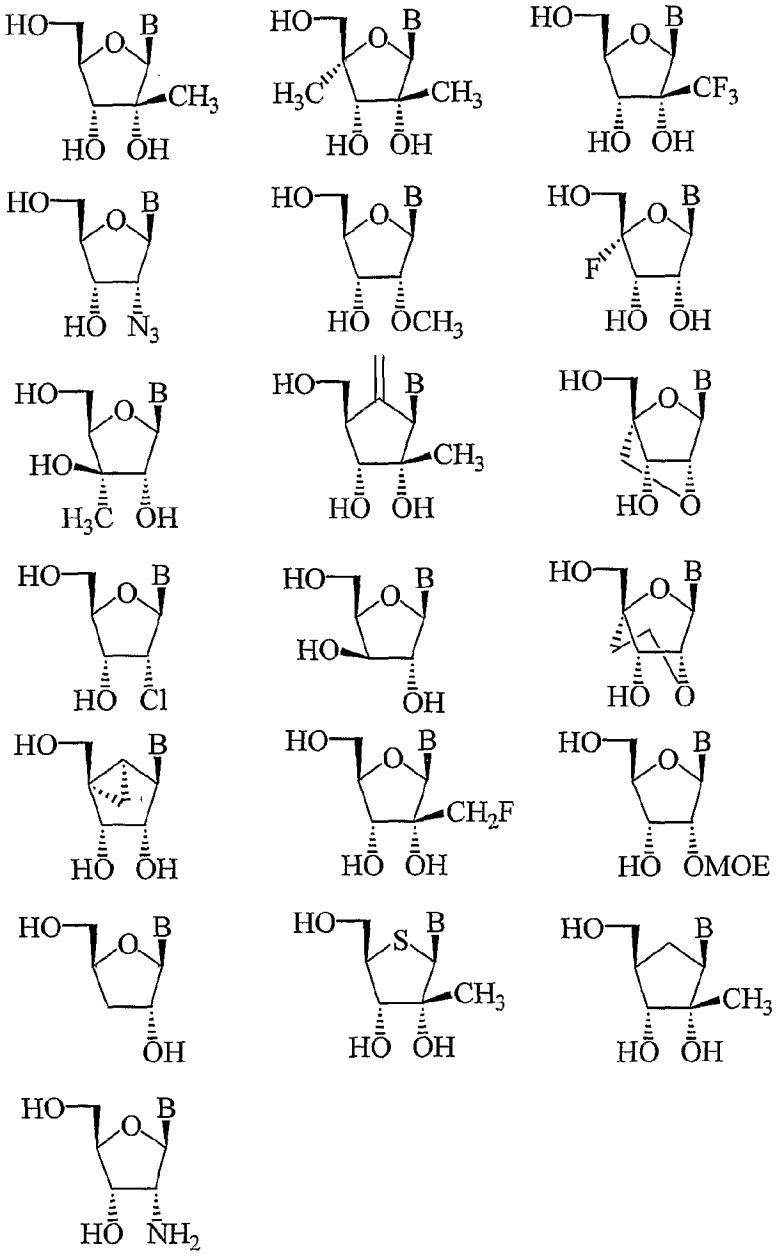
In one aspect of the present invention oligomeric compounds include nucleosides synthetically modified to induce a 3'-endo sugar conformation. A nucleoside can incorporate synthetic modifications of the heterocyclic base, the sugar moiety or both to induce a desired 3'-endo sugar conformation. These modified nucleosides are used to mimic RNA-like nucleosides so that particular properties of an oligomeric compound can be enhanced while maintaining the desirable 3'-endo conformational geometry (see Scheme 1). There is an apparent preference for an RNA type duplex (A form helix, predominantly 3'-endo) as a requirement (e.g. trigger) of RNA interference which is supported in part by the fact that duplexes composed of 2'-deoxy-2'-F-nucleosides appears efficient in triggering RNAi response in the *C. elegans* system. Properties that are enhanced by using more stable 3'-endo nucleosides include but aren't limited to modulation of pharmacokinetic properties through modification of protein binding, protein off-rate, absorption and clearance; modulation of nuclease stability as well as chemical stability; modulation of the binding affinity and specificity of the oligomer (affinity and specificity for enzymes as well as for complementary sequences); and increasing efficacy of RNA cleavage. The present invention provides oligomeric compounds designed to act as triggers of RNAi having one or more nucleosides modified in such a way as to favor a C3'-endo type conformation.

Scheme 1



C2'-endo/Southern C3'-endo/Northern

Along similar lines, oligomeric triggers of RNAi response might be composed of one or more nucleosides modified in such a way that conformation is locked into a C3'-endo type conformation, i.e. Locked Nucleic Acid (LNA, Singh et al, Chem. Commun. (1998), 4, 455-456), and ethylene bridged Nucleic Acids (ENA, Morita et al, Bioorganic & Medicinal Chemistry Letters (2002), 12, 73-76.) Examples of modified nucleosides amenable to the present invention are shown below. These examples are meant to be representative and not exhaustive.

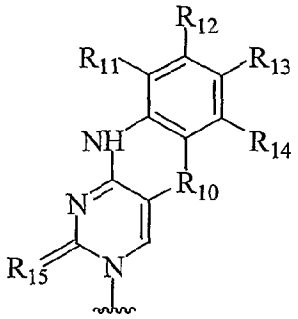


Oligomeric compounds may also include nucleobase (often referred to in the art simply as "base" or "heterocyclic base moiety") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases also referred herein as heterocyclic base moieties include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C≡C-CH_3) uracil and cytosine and other alkynyl

derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

Heterocyclic base moieties may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Some nucleobases include those disclosed in U.S. Patent No. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J.I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., *Angewandte Chemie*, International Edition, 1991, *30*, 613, and those disclosed by Sanghvi, Y.S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Crooke, S.T. and Lebleu, B. , ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2 aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds., *Antisense Research and Applications*, CRC Press, Boca Raton, **1993**, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

In one aspect of the present invention oligomeric compounds are prepared having polycyclic heterocyclic compounds in place of one or more heterocyclic base moieties. A number of tricyclic heterocyclic compounds have been previously reported. These compounds are routinely used in antisense applications to increase the binding properties of the modified strand to a target strand. The most studied modifications are targeted to guanosines hence they have been termed G-clamps or cytidine analogs. Many of these polycyclic heterocyclic compounds have the general formula:



Representative cytosine analogs that make 3 hydrogen bonds with a guanosine in a second strand include 1,3-diazaphenoxazine-2-one (R_{10}= O, R_{11} - R_{14}= H) (Kurchavov, *et al.*, *Nucleosides and Nucleotides*, 1997, 16, 1837-1846), 1,3-diazaphenothiazine-2-one (R_{10}= S, R_{11} - R_{14}= H), (Lin, K.-Y.; Jones, R. J.; Matteucci, M. J. Am. Chem. Soc. 1995, 117, 3873-3874) and 6,7,8,9-tetrafluoro-1,3-diazaphenoxazine-2-one (R_{10} = O, R_{11} - R_{14} = F) (Wang, J.; Lin, K.-Y., Matteucci, M. Tetrahedron Lett. 1998, 39, 8385-8388). When incorporated into oligonucleotides, these base modifications were shown to hybridize with complementary guanine and the latter was also shown to hybridize with adenine and to enhance helical thermal stability by extended stacking interactions (also see U.S. Patent Application Publication 20030207804 and U.S. Patent Application Publication 20030175906, both of which are incorporated herein by reference in their entirety).

Helix-stabilizing properties have been observed when a cytosine analog/substitute has an aminoethoxy moiety attached to the rigid 1,3-diazaphenoxazine-2-one scaffold (R_{10}= O, R_{11} = -O-$(CH_2)_2$-NH_2, R_{12-14}=H) (Lin, K.-Y.; Matteucci, M. J. Am. Chem. Soc. 1998, 120, 8531-8532). Binding studies demonstrated that a single incorporation could enhance the binding affinity of a model oligonucleotide to its complementary target DNA or RNA with a ΔT_m of up to 18° relative to 5-methyl cytosine ($dC5^{me}$), which is the highest known affinity enhancement for a single modification. On the other hand, the gain in helical stability does not compromise the specificity of the oligonucleotides. The T_m data indicate an even greater discrimination between the perfect match and mismatched sequences compared to $dC5^{me}$. It was suggested that the tethered amino group serves as an additional hydrogen bond donor to interact with the Hoogsteen face, namely the O6, of a complementary guanine thereby forming 4 hydrogen bonds. This means that the increased affinity of G-clamp is mediated by the combination of extended base stacking and additional specific hydrogen bonding.

Tricyclic heterocyclic compounds and methods of using them that are amenable to the present invention are disclosed in U.S. Patent 6,028,183, and U.S. Patent 6,007,992, the contents of both are incorporated herein in their entirety.

The enhanced binding affinity of the phenoxazine derivatives together with their sequence specificity makes them valuable nucleobase analogs for the development of more potent antisense-based drugs. In fact, promising data have been derived from *in vitro* experiments demonstrating that heptanucleotides containing phenoxazine substitutions can activate RNaseH, enhance cellular uptake and exhibit an increased antisense activity (Lin, K-Y; Matteucci, M. J. Am. Chem. Soc. 1998, 120, 8531-8532). The activity enhancement was even more pronounced in case of G-clamp, as a single substitution was shown to significantly improve the *in vitro* potency of a 20mer 2'-deoxyphosphorothioate oligonucleotides (Flanagan, W. M.; Wolf, J.J.; Olson, P.; Grant, D.; Lin, K.-Y.; Wagner, R. W.; Matteucci, M. Proc. Natl. Acad. Sci. USA, 1999, 96, 3513-3518).

Modified polycyclic heterocyclic compounds useful as heterocyclic bases are disclosed in but not limited to, the above noted U.S. 3,687,808, as well as U.S.: 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,434,257; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,645,985; 5,646,269; 5,750,692; 5,830,653; 5,763,588; 6,005,096; and 5,681,941, and U.S. Patent Application Publication 20030158403, each of which is incorporated herein by reference in its entirety.

One substitution that can be appended to the oligomeric compounds of the invention involves the linkage of one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the resulting oligomeric compounds. In one embodiment such modified oligomeric compounds are prepared by covalently attaching conjugate groups to functional groups such as hydroxyl or amino groups. Conjugate groups of the invention include intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, polyethers, groups that enhance the pharmacodynamic properties of oligomers, and groups that enhance the pharmacokinetic properties of oligomers. Typical conjugates groups include cholesterols, carbohydrates, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes. Groups that enhance the pharmacodynamic properties, in the context of this invention, include groups that improve oligomer uptake, enhance oligomer resistance to degradation, and/or strengthen hybridization with RNA. Groups that enhance the pharmacokinetic properties, in the context of this invention, include groups that improve oligomer uptake, distribution, metabolism or excretion.

Representative conjugate groups are disclosed in International Patent Application PCT/US92/09196, filed October 23, 1992 the entire disclosure of which is incorporated herein by reference. Conjugate moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556), cholic acid (Manoharan et al., Bioorg. Med. Chem. Let., 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10, 1111-1118; Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937).

The oligomeric compounds of the invention may also be conjugated to active drug substances, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fenbufen, ketoprofen, (*S*)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triiodobenzoic acid, flufenamic acid, folinic acid, a benzothiadiazide, chlorothiazide, a diazepine, indomethicin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic. Oligonucleotide-drug conjugates and their preparation are described in U.S. Patent Application 09/334,130 (filed June 15, 1999) which is incorporated herein by reference in its entirety.

Representative U.S. patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S.: 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference.

Oligomeric compounds used in the compositions of the present invention can also be modified to have one or more stabilizing groups that are generally attached to one or both termini of oligomeric compounds to enhance properties such as for example nuclease stability. Included in stabilizing groups are cap structures. By "cap structure or terminal cap moiety" is meant chemical modifications, which have been incorporated at either terminus of oligonucleotides (see for example Wincott et al., WO 97/26270, incorporated by reference herein). These terminal modifications protect the oligomeric compounds having terminal nucleic acid molecules from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap) or at the 3'-terminus (3'-cap) or can be present on both termini. For double-stranded oligomeric compounds, the cap may be present at either or both termini of either strand. In non-limiting examples, the 5'-cap includes inverted abasic residue (moiety), 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide, carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; threo-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl riucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety (see Wincott et al., International PCT publication No. WO 97/26270, incorporated by reference herein).

Particularly preferred 3'-cap structures of the present invention include, for example 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate, 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; threo-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Tyer, 1993, Tetrahedron 49, 1925; incorporated by reference herein).

Further 3' and 5'-stabilizing groups that can be used to cap one or both ends of an oligomeric compound to impart nuclease stability include those disclosed in WO 03/004602 published on January 16, 2003.

It is not necessary for all positions in an oligomeric compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single oligomeric compound or even at a single monomeric subunit such as a nucleoside within a oligomeric compound. The present invention also includes oligomeric compounds which are chimeric oligomeric compounds. "Chimeric" oligomeric compounds or "chimeras," in the context of this invention, are oligomeric compounds that contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of a nucleic acid based oligomer.

Chimeric oligomeric compounds typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligomeric compound may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, an oligomeric compound may be designed to comprise a region that serves as a substrate for RNase H. RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H by an oligomeric compound having a cleavage region, therefore, results in cleavage of the RNA target, thereby enhancing the efficiency of the oligomeric compound. Consequently, comparable results can often be obtained with shorter oligomeric compounds having substrate regions when chimeras are used, compared to for example phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

Chimeric oligomeric compounds of the invention may be formed as composite structures of two or more oligonucleotides, oligonucleotide mimics, oligonucleotide analogs, oligonucleosides and/or oligonucleotide mimetics as described above. Such oligomeric compounds have also been referred to in the art as hybrids, hemimers, gapmers or inverted gapmers. Representative U.S. patents that teach the preparation of such hybrid structures include, but are not limited to, U.S.: 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, each of which is herein incorporated by reference in its entirety.

The conformation of modified nucleosides and their oligomers can be estimated by various methods such as molecular dynamics calculations, nuclear magnetic resonance

spectroscopy and CD measurements. Hence, modifications predicted to induce RNA-like conformations (A-form duplex geometry in an oligomeric context), are useful in the oligomeric compounds of the present invention. The synthesis of modified nucleosides amenable to the present invention are known in the art (see for example, Chemistry of Nucleosides and Nucleotides Vol 1-3, ed. Leroy B. Townsend, 1988, Plenum Press.)

In one aspect, the present invention is directed to oligomeric compounds that are designed to have enhanced properties compared to native RNA. One method to design optimized or enhanced oligomeric compounds involves each nucleoside of the selected sequence being scrutinized for possible enhancing modifications. One modification would be the replacement of one or more RNA nucleosides with nucleosides that have the same 3'-endo conformational geometry. Such modifications can enhance chemical and nuclease stability relative to native RNA while at the same time being much cheaper and easier to synthesize and/or incorporate into an oligonucleotide. The sequence can be further divided into regions and the nucleosides of each region evaluated for enhancing modifications that can be the result of a chimeric configuration. Consideration is also given to the 5' and 3'-termini as there are often advantageous modifications that can be made to one or more of the terminal nucleosides. The oligomeric compounds of the present invention may include at least one 5'-modified phosphate group on a single strand or on at least one 5'-position of a double-stranded sequence or sequences. Other modifications considered are internucleoside linkages, conjugate groups, substitute sugars or bases, substitution of one or more nucleosides with nucleoside mimetics and any other modification that can enhance the desired property of the oligomeric compound.

One synthetic 2'-modification that imparts increased nuclease resistance and a very high binding affinity to nucleotides is the 2-methoxyethoxy (2'-MOE, 2'-$OCH_2CH_2OCH_3$) side chain (Baker et al., J. Biol. Chem., 1997, 272, 11944-12000). One of the immediate advantages of the 2'-MOE substitution is the improvement in binding affinity, which is greater than many similar 2' modifications such as *O*-methyl, *O*-propyl, and *O*-aminopropyl. Oligonucleotides having the 2'-*O*-methoxyethyl substituent also have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (Martin, P., Helv. Chim. Acta, 1995, 78, 486-504; Altmann et al., Chimia, 1996, 50, 168-176; Altmann et al., Biochem. Soc. Trans., 1996, 24, 630-637; and Altmann et al., Nucleosides Nucleotides, 1997, 16, 917-926). Relative to DNA, the oligonucleotides having the 2'-MOE modification displayed improved RNA affinity and higher nuclease resistance. Chimeric oligonucleotides having 2'-MOE substituents in the wing nucleosides and an internal region of deoxy-phosphorothioate nucleotides (also termed a gapped oligonucleotide or gapmer) have shown effective reduction in the growth of tumors in animal

models at low doses. 2'-MOE substituted oligonucleotides have also shown outstanding promise as antisense agents in several disease states. One such MOE substituted oligonucleotide is presently being investigated in clinical trials for the treatment of CMV retinitis.

Unless otherwise defined herein, alkyl means C_1-C_{12}, C_1-C_8, or C_1-C_6, straight or (where possible) branched chain aliphatic hydrocarbyl.

Unless otherwise defined herein, heteroalkyl means C_1-C_{12}, C_1-C_8, or C_1-C_6, straight or (where possible) branched chain aliphatic hydrocarbyl containing at least one, or about 1 to about 3 hetero atoms in the chain, including the terminal portion of the chain. Suitable heteroatoms include N, O and S.

Unless otherwise defined herein, cycloalkyl means C_3-C_{12}, C_3-C_8, or C_3-C_6, aliphatic hydrocarbyl ring.

Unless otherwise defined herein, alkenyl means C_2-C_{12}, C_2-C_8, or C_2-C_6 alkenyl, which may be straight or (where possible) branched hydrocarbyl moiety, which contains at least one carbon-carbon double bond.

Unless otherwise defined herein, alkynyl means C_2-C_{12}, C_2-C_8, or C_2-C_6 alkynyl, which may be straight or (where possible) branched hydrocarbyl moiety, which contains at least one carbon-carbon triple bond.

Unless otherwise defined herein, heterocycloalkyl means a ring moiety containing at least three ring members, at least one of which is carbon, and of which 1, 2 or three ring members are other than carbon. The number of carbon atoms can vary from 1 to about 12, from 1 to about 6, and the total number of ring members varies from three to about 15, or from about 3 to about 8. Suitable ring heteroatoms are N, O and S. Suitable heterocycloalkyl groups include, but are not limited to, morpholino, thiomorpholino, piperidinyl, piperazinyl, homopiperidinyl, homopiperazinyl, homomorpholino, homothiomorpholino, pyrrolodinyl, tetrahydrooxazolyl, tetrahydroimidazolyl, tetrahydrothiazolyl, tetrahydroisoxazolyl, tetrahydropyrrazolyl, furanyl, pyranyl, and tetrahydroisothiazolyl.

Unless otherwise defined herein, aryl means any hydrocarbon ring structure containing at least one aryl ring. Suitable aryl rings have about 6 to about 20 ring carbons. Especially suitable aryl rings include phenyl, napthyl, anthracenyl, and phenanthrenyl.

Unless otherwise defined herein, hetaryl means a ring moiety containing at least one fully unsaturated ring, the ring consisting of carbon and non-carbon atoms. The ring system can contain about 1 to about 4 rings. The number of carbon atoms can vary from 1 to about 12, from 1 to about 6, and the total number of ring members varies from three to about 15, or from about 3 to about 8. Suitable ring heteroatoms are N, O and S. Suitable hetaryl moieties include, but are

not limited to, pyrazolyl, thiophenyl, pyridyl, imidazolyl, tetrazolyl, pyridyl, pyrimidinyl, purinyl, quinazolinyl, quinoxalinyl, benzimidazolyl, benzothiophenyl, etc.

Unless otherwise defined herein, where a moiety is defined as a compound moiety, such as hetarylalkyl (hetaryl and alkyl), aralkyl (aryl and alkyl), etc., each of the sub-moieties is as defined herein.

Unless otherwise defined herein, an electron withdrawing group is a group, such as the cyano or isocyanato group that draws electronic charge away from the carbon to which it is attached. Other electron withdrawing groups of note include those whose electronegativities exceed that of carbon, for example halogen, nitro, or phenyl substituted in the ortho- or para-position with one or more cyano, isothiocyanato, nitro or halo groups.

Unless otherwise defined herein, the terms halogen and halo have their ordinary meanings. Suitable halo (halogen) substituents are Cl, Br, and I.

The aforementioned optional substituents are, unless otherwise herein defined, suitable substituents depending upon desired properties. Included are halogens (Cl, Br, I), alkyl, alkenyl, and alkynyl moieties, NO_2, NH_3 (substituted and unsubstituted), acid moieties (e.g. $-CO_2H$, $-OSO_3H_2$, etc.), heterocycloalkyl moieties, hetaryl moieties, aryl moieties, etc.

In all the preceding formulae, the squiggle (~) indicates a bond to an oxygen or sulfur of the 5'-phosphate.

Phosphate protecting groups include those described in US Patents No. US 5,760,209, US 5,614,621, US 6,051,699, US 6,020,475, US 6,326,478, US 6,169,177, US 6,121,437, US 6,465,628 each of which is expressly incorporated herein by reference in its entirety.

Screening methods for the identification of effective modulators of small non-coding RNAs are also comprehended by the instant invention and comprise the steps of contacting a small non-coding RNA, or portion thereof, with one or more candidate modulators, and selecting for one or more candidate modulators which decrease or increase the levels, expression or alter the function of the small non-coding RNA. Once it is shown that the candidate modulator or modulators are capable of modulating (e.g. either decreasing or increasing) the levels, expression or altering the function of the small non-coding RNA, the modulator may then be employed in further investigative studies, or for use as a target validation, research, diagnostic, or therapeutic agent in accordance with the present invention.

Screening methods for the identification of small non-coding RNA mimics are also within the scope of the invention. Screening for small non-coding RNA modulators or mimics can also be performed *in vitro*, *ex vivo*, or *in vivo* by contacting samples, tissues, cells or

organisms with candidate modulators or mimics and selecting for one or more candidate modulators which show modulatory effects.

Design and screening of duplexed oligomeric compounds:

In screening and target validation studies, oligomeric compounds of the invention can be used in combination with their respective complementary strand oligomeric compound to form stabilized double-stranded (duplexed) oligonucleotides. In accordance with the present invention, a series of duplexes comprising the oligomeric compounds of the present invention and their complements can be designed to target a small non-coding RNA. The ends of the strands may be modified by the addition of one or more natural or modified nucleobases to form an overhang. The sense strand of the dsRNA is then designed and synthesized as the complement of the antisense strand and may also contain modifications or additions to either terminus. For example, in some embodiments, both strands of the duplex would be complementary over the central nucleobases, each having overhangs at one or both termini, as described *supra*.

In some embodiments, a duplex comprising an antisense strand having the sequence CGAGAGGCGGACGGGACCG (SEQ ID NO:2181) may be prepared with blunt ends (no single stranded overhang) as shown:

```
cgagaggcggacgggaccg     Antisense Strand (SEQ ID NO:2181)
||||||||||||||||||||
gctctccgcctgccctggc     Complement (SEQ ID NO:2184)
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In other embodiments, a duplex comprising an antisense strand having the sequence CGAGAGGCGGACGGGACCG, having a two-nucleobase overhang of deoxythymidine (dT) and its complement sense strand may be prepared with overhangs as shown:

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  cgagaggcggacgggaccgTT     Antisense  Strand (SEQ ID NO:2182)
  ||||||||||||||||||||
TTgctctccgcctgccctggc        Complement Sense Strand (SEQ ID NO:2183)
```

RNA strands of the duplex can be synthesized by methods disclosed herein or purchased from Dharmacon Research Inc., (Lafayette, CO).

For use in drug discovery, oligomeric compounds of the present invention are used to elucidate relationships that exist between small non-coding RNAs, genes or proteins and a disease state, phenotype, or condition. These methods include detecting or modulating a target comprising contacting a sample, tissue, cell, or organism with the oligomeric compounds and compositions of the present invention, measuring the levels of the target and/or the levels of downstream gene products including mRNA or proteins encoded thereby, a related phenotypic or chemical endpoint at some time after treatment, and optionally comparing the measured value to

an untreated sample, a positive control or a negative control. These methods can also be performed in parallel or in combination with other experiments to determine the function of unknown genes for the process of target validation or to determine the validity of a particular gene product as a target for treatment or prevention of a disease.

The oligomeric compounds and compositions of the present invention can additionally be utilized for diagnostics, therapeutics, prophylaxis and as research reagents and kits. Such uses allows for those of ordinary skill to elucidate the function of particular non-coding or coding nucleic acids or to distinguish between functions of various members of a biological pathway.

For use in kits and diagnostics, the oligomeric compounds and compositions of the present invention, either alone or in combination with other compounds or therapeutics, can be used as tools in differential and/or combinatorial analyses to elucidate expression patterns of a portion or the entire complement of non-coding or coding nucleic acids expressed within cells and tissues.

As one non-limiting example, expression patterns within cells or tissues treated with one or more oligomeric compounds or compositions of the invention are compared to control cells or tissues not treated with the compounds or compositions and the patterns produced are analyzed for differential levels of nucleic acid expression as they pertain, for example, to disease association, signaling pathway, cellular localization, expression level, size, structure or function of the genes examined. These analyses can be performed on stimulated or unstimulated cells and in the presence or absence of other compounds that affect expression patterns.

Cell culture and oligonucleotide treatment:

The effects of oligomeric compounds on target nucleic acid expression or function can be tested in any of a variety of cell types provided that the target nucleic acid is present at measurable levels. This can be readily determined by methods routine in the art, for example Northern blot analysis, ribonuclease protection assays, or real-time RT-PCR. The following cell types are provided for illustrative purposes, but other cell types can be routinely used, provided that the target is present in the cell type chosen.

T-24 cells:

The human transitional cell bladder carcinoma cell line T-24 is obtained from the American Type Culture Collection (ATCC) (Manassas, VA). T-24 cells were routinely cultured in complete McCoy's 5A basal media (Invitrogen Corporation, Carlsbad, CA) supplemented with 10% fetal calf serum (Invitrogen Corporation, Carlsbad, CA), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Invitrogen Corporation, Carlsbad, CA). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. For

Northern blotting or other analyses, cells harvested when they reached 90% confluence. Cells were seeded into 96-well plates (Falcon-Primaria #353872) at a density of 7000 cells/well for use in real-time RT-PCR analysis.

A549 cells:

The human lung carcinoma cell line A549 is obtained from the American Type Culture Collection (ATCC) (Manassas, VA). A549 cells were routinely cultured in DMEM basal media (Invitrogen Corporation, Carlsbad, CA) supplemented with 10% fetal calf serum (Invitrogen Corporation, Carlsbad, CA), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Invitrogen Corporation, Carlsbad, CA). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence.

HMECs:

Normal human mammary epithelial cells (HMECs) are obtained from American Type Culture Collection (Manassus, VA). HMECs are routinely cultured in DMEM high glucose (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA). Cells are routinely passaged by trypsinization and dilution when they reach approximately 90% confluence. HMECs are plated in 24-well plates (Falcon-Primaria # 353047, BD Biosciences, Bedford, MA) at a density of 50,000-60,000 cells per well, and allowed to attach overnight prior to treatment with oligomeric compounds. HMECs are plated in 96-well plates (Falcon-Primaria #353872, BD Biosciences, Bedford, MA) at a density of approximately 10,000 cells per well and allowed to attach overnight prior to treatment with oligomeric compounds.

MCF7 cells:

The breast carcinoma cell line MCF7 is obtained from American Type Culture Collection (Manassus, VA). MCF7 cells are routinely cultured in DMEM high glucose (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA). Cells are routinely passaged by trypsinization and dilution when they reach approximately 90% confluence. MCF7 cells are plated in 24-well plates (Falcon-Primaria # 353047, BD Biosciences, Bedford, MA) at a density of approximately 140,000 cells per well, and allowed to attach overnight prior to treatment with oligomeric compounds. MCF7 cells are plated in 96-well plates (Falcon-Primaria #353872, BD Biosciences, Bedford, MA) at a density of approximately 20,000 cells per well and allowed to attach overnight prior to treatment with oligomeric compounds.

T47D cells:

The breast carcinoma cell line T47D is obtained from American Type Culture

Collection (Manassus, VA). T47D cells are deficient in expression of the tumor suppressor gene p53. T47D cells are cultured in DMEM high glucose (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA). Cells are routinely passaged by trypsinization and dilution when they reach approximately 90% confluence. T47D cells are plated in 24-well plates (Falcon-Primaria # 353047, BD Biosciences, Bedford, MA) at a density of approximately 170,000 cells per well, and allowed to attach overnight prior to treatment with oligomeric compounds. T47D cells are plated in 96-well plates (Falcon-Primaria #353872, BD Biosciences, Bedford, MA) at a density of approximately 20,000 cells per well and allowed to attach overnight prior to treatment with oligomeric compounds.

BJ cells:

The normal human foreskin fibroblast BJ cell line was obtained from American Type Culture Collection (Manassus, VA). BJ cells were routinely cultured in MEM high glucose with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate and supplemented with 10 % fetal bovine serum, 0.1 mM non-essential amino acids and 1.0 mM sodium pyruvate (all media and supplements from Invitrogen Life Technologies, Carlsbad, CA). Cells were routinely passaged by trypsinization and dilution when they reached approximately 80% confluence. Cells were plated on collagen-coated 24-well plates (Falcon-Primaria #3047, BD Biosciences, Bedford, MA) at approximately 50,000 cells per well, and allowed to attach to wells overnight.

B16-F10 cells:

The mouse melanoma cell line B16-F10 was obtained from American Type Culture Collection (Manassas, VA). B16-F10 cells were routinely cultured in DMEM high glucose (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA). Cells were routinely passaged by trypsinization and dilution when they reached approximately 80% confluence. Cells were seeded into collagen-coated 24-well plates (Falcon-Primaria #3047, BD Biosciences, Bedford, MA) at approximately 50,000 cells per well and allowed to attach overnight.

HUVECs:

Human vascular endothelial cells (HUVECs) are obtained from American Type Culture Collection (Manassus, VA). HUVECs are routinely cultured in EBM (Clonetics Corporation, Walkersville, MD) supplemented with SingleQuots supplements (Clonetics Corporation, Walkersville, MD). Cells are routinely passaged by trypsinization and dilution when they reach approximately 90% confluence and are maintained for up to 15 passages. HUVECs are plated at approximately 3000 cells/well in 96-well plates (Falcon-Primaria #353872, BD Biosciences,

Bedford, MA) and treated with oligomeric compounds one day later.

NHDF cells:

Human neonatal dermal fibroblast (NHDF) cells are obtained from the Clonetics Corporation (Walkersville, MD). NHDFs were routinely maintained in Fibroblast Growth Medium (Clonetics Corporation, Walkersville, MD) supplemented as recommended by the supplier. Cells were maintained for up to 10 passages as recommended by the supplier.

HEK cells:

Human embryonic keratinocytes (HEK) are obtained from the Clonetics Corporation (Walkersville, MD). HEKs were routinely maintained in Keratinocyte Growth Medium (Clonetics Corporation, Walkersville, MD) formulated as recommended by the supplier. Cells were routinely maintained for up to 10 passages as recommended by the supplier.

293T cells:

The human 293T cell line is obtained from American Type Culture Collection (Manassas, VA). 293T cells are a highly transfectable cell line constitutively expressing the simian virus 40 (SV40) large T antigen. 293T cells were maintained in Dulbeccos' Modified Medium (DMEM) (Invitrogen Corporation, Carlsbad, CA) supplemented with 10% fetal calf serum and antibiotics (Life Technologies).

HepG2 cells:

The human hepatoblastoma cell line HepG2 is obtained from the American Type Culture Collection (ATCC) (Manassas, VA). HepG2 cells are routinely cultured in Eagle's MEM supplemented with 10% fetal bovine serum, 1 mM non-essential amino acids, and 1 mM sodium pyruvate (medium and all supplements from Invitrogen Life Technologies, Carlsbad, CA). Cells are routinely passaged by trypsinization and dilution when they reach approximately 90% confluence. For treatment with oligomeric compounds, cells are seeded into 96-well plates (Falcon-Primaria #353872, BD Biosciences, Bedford, MA) at a density of approximately 7000 cells/well prior to treatment with oligomeric compounds. For the caspase assay, cells are seeded into collagen coated 96-well plates (BIOCOAT cellware, Collagen type I, B-D #354407/356407, Becton Dickinson, Bedford, MA) at a density of 7500 cells/well.

Preadipocytes:

Human preadipocytes are obtained from Zen-Bio, Inc. (Research Triangle Park, NC). Preadipocytes were routinely maintained in Preadipocyte Medium (ZenBio, Inc., Research Triangle Park, NC) supplemented with antibiotics as recommended by the supplier. Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells were routinely maintained for up to 5 passages as recommended by the supplier. To induce

differentiation of preadipocytes, cells are then incubated with differentiation media consisting of Preadipocyte Medium further supplemented with 2% more fetal bovine serum (final total of 12%), amino acids, 100 nM insulin, 0.5 mM IBMX, 1 μM dexamethasone and 1 μM BRL49653. Cells are left in differentiation media for 3-5 days and then re-fed with adipocyte media consisting of Preadipocyte Medium supplemented with 33 μM biotin, 17 μM pantothenate, 100 nM insulin and 1 μM dexamethasone. Cells differentiate within one week. At this point cells are ready for treatment with the oligomeric compounds of the invention. One day prior to transfection, 96-well plates (Falcon-Primaria #353872, BD Biosciences, Bedford, MA) are seeded with approximately 3000 cells/well prior to treatment with oligomeric compounds.

Differentiated adipocytes:

Human adipocytes are obtained from Zen-Bio, Inc. (Research Triangle Park, NC). Adipocytes were routinely maintained in Adipocyte Medium (ZenBio, Inc., Research Triangle Park, NC) supplemented with antibiotics as recommended by the supplier. Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells were routinely maintained for up to 5 passages as recommended by the supplier.

NT2 cells:

The NT2 cell line is obtained from the American Type Culture Collection (ATCC; Manassa, VA). The NT2 cell line, which has the ATCC designation NTERA-2 cl.D1, is a pluripotent human testicular embryonal carcinoma cell line derived by cloning the NTERA-2 cell line. The parental NTERA-2 line was established in 1980 from a nude mouse xenograft of the Tera-2 cell line (ATCC HTB-106). NT2 cells were routinely cultured in DMEM, high glucose (Invitrogen Corporation, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen Corporation, Carlsbad, CA). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. For Northern blotting or other analyses, cells harvested when they reached 90% confluence.

HeLa cells:

The human epitheloid carcinoma cell line HeLa is obtained from the American Tissue Type Culture Collection (Manassas, VA). HeLa cells were routinely cultured in DMEM, high glucose (Invitrogen Corporation, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen Corporation, Carlsbad, CA). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. For Northern blotting or other analyses, cells were harvested when they reached 90% confluence.

For Northern blotting or other analysis, cells may be seeded onto 100 mm or other standard tissue culture plates and treated similarly, using appropriate volumes of medium and oligonucleotide.

Treatment with antisense oligomeric compounds:

In general, when cells reach approximately 80% confluency, they are treated with oligomeric compounds of the invention. Oligomeric compounds are introduced into cells using the cationic lipid transfection reagent LIPOFECTIN™ (Invitrogen Life Technologies, Carlsbad, CA). Oligomeric compounds are mixed with LIPOFECTIN™ in OPTI-MEM™ (Invitrogen Life Technologies, Carlsbad, CA) to achieve the desired final concentration of oligomeric compound and LIPOFECTIN™. Before adding to cells, the oligomeric compound, LIPOFECTIN™ and OPTI-MEM™ are mixed thoroughly and incubated for approximately 0.5 hrs. The medium is removed from the plates and the plates are tapped on sterile gauze. Each well of a 96-well plate is washed with 150 µl of phosphate-buffered saline or Hank's balanced salt solution. Each well of a 24-well plate is washed with 250 µL of phosphate-buffered saline or Hank's balanced salt solution. The wash buffer in each well is replaced with 100 µL or 250 µL of the oligomeric compound/OPTI-MEM™/LIPOFECTIN™ cocktail for 96-well or 24-well plates, respectively. Untreated control cells receive LIPOFECTIN™ only. The plates are incubated for approximately 4 to 7 hours at 37°C, after which the medium is removed and the plates are tapped on sterile gauze. 100 µl or 1 mL of full growth medium is added to each well of a 96-well plate or a 24-well plate, respectively. Cells are harvested 16-24 hours after oligonucleotide treatment, at which time RNA can be isolated and target reduction measured by real-time RT-PCR, or other phenotypic assays performed. In general, data from treated cells are obtained in triplicate, and results presented as an average of the three trials.

In some embodiments, cells are transiently transfected with oligomeric compounds of the instant invention. In some embodiments, cells are transfected and selected for stable expression of an oligomeric compound of the instant invention.

The concentration of oligonucleotide used varies from cell line to cell line. To determine the optimal oligonucleotide concentration for a particular cell line, the cells are treated with a positive control oligonucleotide at a range of concentrations. For human cells the positive control oligonucleotide may be selected from ISIS 13920 (**TCC**GTCATCGCT**CCTCAGGG**, SEQ ID NO: 1) which is targeted to human H-ras, or ISIS 18078, (**GTGCG**CGCGAGCCCG**AAATC**, SEQ ID NO: 2) which is targeted to human Jun-N-terminal kinase-2 (JNK2) or another suitable positive control. Controls are 2'-O-methoxyethyl gapmers

(2'-O-methoxyethyls shown in bold) with a phosphorothioate backbone or having chemical modifications similar to the oligonucleotides being tested. For mouse or rat cells the positive control oligonucleotide may be ISIS 15770 (**ATGCA**TTCTGCCCCC**AAGGA**, SEQ ID NO: 3), a 2'-O-methoxyethyl gapmer (2'-O-methoxyethyls shown in bold) with a phosphorothioate backbone which is targeted to both mouse and rat c-raf. The concentration of positive control oligonucleotide that results in 80% inhibition of c-H-ras (for ISIS 13920), JNK2 (for ISIS 18078) or c-raf (for ISIS 15770) or other suitable control target RNA may then be utilized as the screening concentration for new oligonucleotides in subsequent experiments for that cell line. If 80% inhibition is not achieved, the lowest concentration of positive control oligonucleotide that results in 60% inhibition of target expression or function is then utilized as the oligonucleotide screening concentration in subsequent experiments for that cell line. The concentrations of oligonucleotides used herein can range from 10 nM to 300 nM.

Examples of methods of gene expression analysis known in the art include DNA arrays or microarrays (Brazma and Vilo, FEBS Lett., 2000, 480, 17-24; Celis, et al., FEBS Lett., 2000, 480, 2-16), SAGE (serial analysis of gene expression)(Madden, et al., Drug Discov. Today, 2000, 5, 415-425), READS (restriction enzyme amplification of digested cDNAs) (Prashar and Weissman, Methods Enzymol., 1999, 303, 258-72), TOGA (total gene expression analysis) (Sutcliffe, et al., Proc. Natl. Acad. Sci. U. S. A., 2000, 97, 1976-81), protein arrays and proteomics (Celis, et al., FEBS Lett., 2000, 480, 2-16; Jungblut, et al., Electrophoresis, 1999, 20, 2100-10), expressed sequence tag (EST) sequencing (Celis, et al., FEBS Lett., 2000, 480, 2-16; Larsson, et al., J. Biotechnol., 2000, 80, 143-57), subtractive RNA fingerprinting (SuRF) (Fuchs, et al., Anal. Biochem., 2000, 286, 91-98; Larson, et al., Cytometry, 2000, 41, 203-208), subtractive cloning, differential display (DD) (Jurecic and Belmont, Curr. Opin. Microbiol., 2000, 3, 316-21), comparative genomic hybridization (Carulli, et al., J. Cell Biochem. Suppl., 1998, 31, 286-96), FISH (fluorescent in situ hybridization) techniques (Going and Gusterson, Eur. J. Cancer, 1999, 35, 1895-904), mass spectrometry methods (To, Comb. Chem. High Throughput Screen, 2000, 3, 235-41) and real-time quantitative RT-PCR (Heid, et al., Genome Res., 1996, 6(10), 986-94).

Analysis of oligonucleotide inhibition of a target levels or expression:

Modulation of target levels or expression can be assayed in a variety of ways known in the art. For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or real-time quantitative RT-PCR (also known as RT-PCR). Real-time quantitative RT-PCR is presently preferred. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well

known in the art. Northern blot analysis is also routine in the art. Real-time quantitative RT-PCR can be conveniently accomplished using the commercially available ABI PRISM™ 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

RNA Isolation:

Poly(A)+ mRNA isolation

Poly(A)+ mRNA was isolated according to Miura *et al.*, (*Clin. Chem.*, **1996**, *42*, 1758-1764). Other methods for poly(A)+ mRNA isolation are routine in the art. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200 µL cold phosphate-buffered saline (PBS). 60 µL lysis buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA, 0.5 M NaCl, 0.5% NP-40, 20 mM vanadyl-ribonucleoside complex) was added to each well, the plate was gently agitated and then incubated at room temperature for five minutes. 55 µL of lysate was transferred to Oligo d(T) coated 96-well plates (AGCT Inc., Irvine CA). Plates were incubated for 60 minutes at room temperature, washed 3 times with 200 µL of wash buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA, 0.3 M NaCl). After the final wash, the plate was blotted on paper towels to remove excess wash buffer and then air-dried for 5 minutes. 60 µL of elution buffer (5 mM Tris-HCl pH 7.6), preheated to 70°C, was added to each well, the plate was incubated on a 90°C hot plate for 5 minutes, and the eluate was then transferred to a fresh 96-well plate.

Cells grown on 100 mm or other standard plates may be treated similarly, using appropriate volumes of all solutions.

Total RNA Isolation

Total RNA was isolated using an RNEASY 96™ kit and buffers purchased from Qiagen Inc. (Valencia, CA) following the manufacturer's recommended procedures. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200 µL cold PBS. 150 µL Buffer RLT was added to each well and the plate vigorously agitated for 20 seconds. 150 µL of 70% ethanol was then added to each well and the contents mixed by pipetting three times up and down. The samples were then transferred to the RNEASY 96™ well plate attached to a QIAVAC™ manifold fitted with a waste collection tray and attached to a vacuum source. Vacuum was applied for 1 minute. 500 µL of Buffer RW1 was added to each well of the RNEASY 96™ plate and incubated for 15 minutes and the vacuum was again applied for 1 minute. An additional 500 µL of Buffer RW1 was added to each well of the RNEASY 96™ plate and the vacuum was applied for 2 minutes. 1 mL of Buffer RPE was then

added to each well of the RNEASY 96™ plate and the vacuum applied for a period of 90 seconds. The Buffer RPE wash was then repeated and the vacuum was applied for an additional 3 minutes. The plate was then removed from the QIAVAC™ manifold and blotted dry on paper towels. The plate was then re-attached to the QIAVAC™ manifold fitted with a collection tube rack containing 1.2 mL collection tubes. RNA was then eluted by pipetting 140 µL of RNAse free water into each well, incubating 1 minute, and then applying the vacuum for 3 minutes.

The repetitive pipetting and elution steps may be automated using a QIAGEN Bio-Robot 9604 (Qiagen, Inc., Valencia CA). Essentially, after lysing of the cells on the culture plate, the plate is transferred to the robot deck where the pipetting, DNase treatment and elution steps are carried out.

Real-time Quantitative PCR Analysis of a target RNA Levels:

Quantitation of a target RNA levels was accomplished by real-time quantitative PCR using the ABI PRISM™ 7600, 7700, or 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to manufacturer's instructions. This is a closed-tube, non-gel-based, fluorescence detection system which allows high-throughput quantitation of polymerase chain reaction (PCR) products in real-time. As opposed to standard PCR in which amplification products are quantitated after the PCR is completed, products in real-time quantitative PCR are quantitated as they accumulate. This is accomplished by including in the PCR reaction an oligonucleotide probe that anneals specifically between the forward and reverse PCR primers, and contains two fluorescent dyes. A reporter dye (e.g., FAM or JOE, obtained from either PE-Applied Biosystems, Foster City, CA, Operon Technologies Inc., Alameda, CA or Integrated DNA Technologies Inc., Coralville, IA) is attached to the 5' end of the probe and a quencher dye (e.g., TAMRA, obtained from either PE-Applied Biosystems, Foster City, CA, Operon Technologies Inc., Alameda, CA or Integrated DNA Technologies Inc., Coralville, IA) is attached to the 3' end of the probe. When the probe and dyes are intact, reporter dye emission is quenched by the proximity of the 3' quencher dye. During amplification, annealing of the probe to the target sequence creates a substrate that can be cleaved by the 5'-exonuclease activity of Taq polymerase. During the extension phase of the PCR amplification cycle, cleavage of the probe by Taq polymerase releases the reporter dye from the remainder of the probe (and hence from the quencher moiety) and a sequence-specific fluorescent signal is generated. With each cycle, additional reporter dye molecules are cleaved from their respective probes, and the fluorescence intensity is monitored at regular intervals by laser optics built into the ABI PRISM™ Sequence Detection System. In each assay, a series of parallel reactions containing

serial dilutions of RNA from untreated control samples generates a standard curve that is used to quantitate the percent inhibition after oligonucleotide treatment of test samples.

Prior to quantitative PCR analysis, primer/probe sets specific to the target gene (or RNA) being measured are evaluated for their ability to be "multiplexed" with a GAPDH amplification reaction. In multiplexing, both the target gene (or RNA) and the internal standard gene GAPDH are amplified concurrently in a single sample. In this analysis, RNA isolated from untreated cells is serially diluted. Each dilution is amplified in the presence of primer/probe sets specific for GAPDH only, target gene (or RNA) only ("single-plexing"), or both (multiplexing). Following PCR amplification, standard curves of GAPDH and target RNA signal as a function of dilution are generated from both the single-plexed and multiplexed samples. If both the slope and correlation coefficient of the GAPDH and target signals generated from the multiplexed samples fall within 10% of their corresponding values generated from the single-plexed samples, the primer/probe set specific for that target is deemed multiplexable. Other methods of PCR are also known in the art.

PCR reagents were obtained from Invitrogen Corporation, (Carlsbad, CA). RT-PCR reactions were carried out by adding 20 µL PCR cocktail (2.5x PCR buffer minus $MgCl_2$, 6.6 mM $MgCl_2$, 375 µM each of dATP, dCTP, dCTP and dGTP, 375 nM each of forward primer and reverse primer, 125 nM of probe, 4 Units RNAse inhibitor, 1.25 Units PLATINUM® Taq, 5 Units MuLV reverse transcriptase, and 2.5x ROX dye) to 96-well plates containing 30 µL total RNA solution (20-200 ng). The RT reaction was carried out by incubation for 30 minutes at 48°C. Following a 10 minute incubation at 95°C to activate the PLATINUM® Taq, 40 cycles of a two-step PCR protocol were carried out: 95°C for 15 seconds (denaturation) followed by 60°C for 1.5 minutes (annealing/extension).

Gene (or RNA) target quantities obtained by real time RT-PCR are normalized using either the expression level of GAPDH, a gene whose expression is constant, or by quantifying total RNA using RiboGreen™ (Molecular Probes, Inc. Eugene, OR). GAPDH expression is quantified by real time RT-PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RiboGreen™ RNA quantification reagent (Molecular Probes, Inc. Eugene, OR). Methods of RNA quantification by RiboGreen™ are taught in Jones, L.J., et al, (Analytical Biochemistry, 1998, 265, 368-374).

In this assay, 170 µL of RiboGreen™ working reagent (RiboGreen™ reagent diluted 1:350 in 10mM Tris-HCl, 1 mM EDTA, pH 7.5) is pipetted into a 96-well plate containing 30

μL purified, cellular RNA. The plate is read in a CytoFluor 4000 (PE Applied Biosystems) with excitation at 485nm and emission at 530nm.

Probes and primers are designed to hybridize to the target sequence.

Northern blot analysis of target RNA levels:

Eighteen hours after treatment, cell monolayers were washed twice with cold PBS and lysed in 1 mL RNAZOL™ (TEL-TEST "B" Inc., Friendswood, TX). Total RNA was prepared following manufacturer's recommended protocols. Twenty micrograms of total RNA was fractionated by electrophoresis through 1.2% agarose gels containing 1.1% formaldehyde using a MOPS buffer system (AMRESCO, Inc. Solon, OH). RNA was transferred from the gel to HYBOND™-N+ nylon membranes (Amersham Pharmacia Biotech, Piscataway, NJ) by overnight capillary transfer using a Northern/Southern Transfer buffer system (TEL-TEST "B" Inc., Friendswood, TX). RNA transfer was confirmed by UV visualization. Membranes were fixed by UV cross-linking using a STRATALINKER™ UV Crosslinker 2400 (Stratagene, Inc, La Jolla, CA) and then probed using QUICKHYB™ hybridization solution (Stratagene, La Jolla, CA) using manufacturer's recommendations for stringent conditions.

To detect a target, a target specific primer/probe set is prepared for analysis by PCR. To normalize for variations in loading and transfer efficiency, membranes can be stripped and probed for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA (Clontech, Palo Alto, CA).

Hybridized membranes were visualized and quantitated using a PHOSPHORIMAGER™ and IMAGEQUANT™ Software V3.3 (Molecular Dynamics, Sunnyvale, CA). Data can be normalized to GAPDH levels in untreated controls.

The compounds and compositions of the invention are useful for research and diagnostics, because these compounds and compositions hybridize to nucleic acids or interfere with the normal function of these nucleic acids. Hybridization of the compounds and compositions of the invention with a nucleic acid can be detected by means known in the art. Such means may include conjugation of an enzyme to the compound or composition, radiolabeling or any other suitable detection means. Kits using such detection means for detecting the level of selected proteins in a sample may also be prepared.

The specificity and sensitivity of compounds and compositions can also be harnessed by those of skill in the art for therapeutic uses. Antisense oligomeric compounds have been employed as therapeutic moieties in the treatment of disease states in animals, including humans. Antisense oligonucleotide drugs, including ribozymes, have been safely and effectively

administered to humans and numerous clinical trials are presently underway. It is thus established that oligomeric compounds can be useful therapeutic modalities that can be configured to be useful in treatment regimes for the treatment of cells, tissues and animals, especially humans.

For therapeutics, an animal, preferably a human, suspected of having a disease or disorder presenting conditions that can be treated, ameliorated, or improved by modulating the expression of a selected small non-coding target nucleic acid is treated by administering the compounds and compositions. For example, in one non-limiting embodiment, the methods comprise the step of administering to or contacting the animal, an effective amount of a modulator or mimic to treat, ameliorate or improve the conditions associated with the disease or disorder. The compounds of the present invention effectively modulate the activity or function of the small non-coding RNA target or inhibit the expression or levels of the small non-coding RNA target. In one embodiment, the activity or expression of the target in an animal is inhibited by about 10%. In another embodiment the activity or expression of a target in an animal is inhibited by about 30%. Further, the activity or expression of a target in an animal is inhibited by 50% or more, by 60% or more, by 70% or more, by 80% or more, by 90% or more, or by 95% or more. In another embodiment, the present invention provides for the use of a compound of the invention in the manufacture of a medicament for the treatment of any and all conditions disclosed herein.

The reduction of target levels may be measured in serum, adipose tissue, liver or any other body fluid, tissue or organ of the animal known to contain the small non-coding RNA or its precursor. Further, the cells contained within the fluids, tissues or organs being analyzed contain a nucleic acid molecule of a downstream target regulated or modulated by the small non-coding RNA target itself.

The oligomeric compounds and compositions of the invention can be utilized in pharmaceutical compositions by adding an effective amount of the compound or composition to a suitable pharmaceutically acceptable diluent or carrier. Use of the oligomeric compounds and methods of the invention may also be useful prophylactically.

The oligomeric compounds and compositions of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor-targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative U.S. patents that teach the preparation of such uptake, distribution and/or absorption-assisting formulations include, but are not limited to, U.S.: 5,108,921; 5,354,844;

5,416,016; 5,459,127; 5,521,291; 5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of which is herein incorporated by reference.

The oligomeric compounds and compositions of the invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to prodrugs and pharmaceutically acceptable salts of the oligomeric compounds of the invention, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents.

The term "prodrug" indicates a therapeutic agent that is prepared in an inactive form that is converted to an active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions. In particular, prodrug versions of the oligomeric compounds of the invention can be prepared as SATE ((S-acetyl-2-thioethyl) phosphate) derivatives according to the methods disclosed in WO 93/24510 to Gosselin *et al.*, published December 9, 1993 or in WO 94/26764 and U.S. 5,770,713 to Imbach *et al.* Larger oligomeric compounds that are processed to supply, as cleavage products, compounds capable of modulating the function or expression of small non-coding RNAs or their downstream targets are also considered prodrugs.

The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds and compositions of the invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. Suitable examples include, but are not limited to, sodium and postassium salts. For oligonucleotides, examples of pharmaceutically acceptable salts and their uses are further described in U.S. Patent 6,287,860, which is incorporated herein in its entirety.

The present invention also includes pharmaceutical compositions and formulations that include the oligomeric compounds and compositions of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular

injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration. Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

Oligomeric compounds may be formulated for delivery *in vivo* in an acceptable dosage form, e.g. as parenteral or non-parenteral formulations. Parenteral formulations include intravenous (IV), subcutaneous (SC), intraperitoneal (IP), intravitreal and intramuscular (IM) formulations, as well as formulations for delivery via pulmonary inhalation, intranasal administration, topical administration, etc. Non-parenteral formulations include formulations for delivery via the alimentary canal, e.g. oral administration, rectal administration, intrajejunal instillation, etc. Rectal administration includes administration as an enema or a suppository. Oral administration includes administration as a capsule, a gel capsule, a pill, an elixir, etc.

In some embodiments, an oligomeric compound can be administered to a subject via an oral route of administration. The subject may be an animal or a human (man). An animal subject may be a mammal, such as a mouse, a rat, a dog, a guinea pig, a monkey, a non-human primate, a cat or a pig. Non-human primates include monkeys and chimpanzees. A suitable animal subject may be an experimental animal, such as a mouse, rat, mouse, a rat, a dog, a monkey, a non-human primate, a cat or a pig.

In some embodiments, the subject may be a human. In certain embodiments, the subject may be a human patient. In certain embodiments, the subject may be in need of modulation of expression of one or more genes as discussed in more detail herein. In some particular embodiments, the subject may be in need of inhibition of expression of one or more genes as discussed in more detail herein. In particular embodiments, the subject may be in need of modulation, i.e. inhibition or enhancement, of a nucleic acid target in order to obtain therapeutic indications discussed in more detail herein.

In some embodiments, non-parenteral (e.g. oral) oligomeric compound formulations according to the present invention result in enhanced bioavailability of the compound. In this context, the term "bioavailability" refers to a measurement of that portion of an administered drug which reaches the circulatory system (e.g. blood, especially blood plasma) when a particular mode of administration is used to deliver the drug. Enhanced bioavailability refers to a particular mode of administration's ability to deliver oligonucleotide to the peripheral blood plasma of a subject relative to another mode of administration. For example, when a non-parenteral mode of administration (e.g. an oral mode) is used to introduce the drug into a subject,

the bioavailability for that mode of administration may be compared to a different mode of administration, e.g. an IV mode of administration. In some embodiments, the area under a compound's blood plasma concentration curve (AUC_0) after non-parenteral (e.g. oral, rectal, intrajejunal) administration may be divided by the area under the drug's plasma concentration curve after intravenous (i.v.) administration (AUC_{iv}) to provide a dimensionless quotient (relative bioavailability, RB) that represents the fraction of compound absorbed via the non-parenteral route as compared to the IV route. A composition's bioavailability is said to be enhanced in comparison to another composition's bioavailability when the first composition's relative bioavailability (RB_1) is greater than the second composition's relative bioavailability (RB_2).

In general, bioavailability correlates with therapeutic efficacy when a compound's therapeutic efficacy is related to the blood concentration achieved, even if the drug's ultimate site of action is intracellular (van Berge-Henegouwen *et al.*, *Gastroenterol.*, 1977, *73,* 300). Bioavailability studies have been used to determine the degree of intestinal absorption of a drug by measuring the change in peripheral blood levels of the drug after an oral dose (DiSanto, Chapter 76 *In: Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990, pages 1451-1458).

In general, an oral composition's bioavailability is said to be "enhanced" when its relative bioavailability is greater than the bioavailability of a composition substantially consisting of pure oligonucleotide, i.e. oligonucleotide in the absence of a penetration enhancer.

Organ bioavailability refers to the concentration of compound in an organ. Organ bioavailability may be measured in test subjects by a number of means, such as by whole-body radiography. Organ bioavailability may be modified, e.g. enhanced, by one or more modifications to the oligomeric compound, by use of one or more carrier compounds or excipients. In general, an increase in bioavailability will result in an increase in organ bioavailability.

Oral oligomeric compound compositions according to the present invention may comprise one or more "mucosal penetration enhancers," also known as "absorption enhancers" or simply as "penetration enhancers." Accordingly, some embodiments of the invention comprise at least one oligomeric compound in combination with at least one penetration enhancer. In general, a penetration enhancer is a substance that facilitates the transport of a drug across mucous membrane(s) associated with the desired mode of administration, e.g. intestinal epithelial membranes. Accordingly it is desirable to select one or more penetration enhancers

that facilitate the uptake of one or more oligomeric compounds, without interfering with the activity of the compounds, and in such a manner the compounds can be introduced into the body of an animal without unacceptable side-effects such as toxicity, irritation or allergic response.

Embodiments of the present invention provide compositions comprising one or more pharmaceutically acceptable penetration enhancers, and methods of using such compositions, which result in the improved bioavailability of oligomeric compounds administered via non-parenteral modes of administration. Heretofore, certain penetration enhancers have been used to improve the bioavailability of certain drugs. See Muranishi, *Crit. Rev. Ther. Drug Carrier Systems*, 1990, *7*, 1 and Lee *et al.*, *Crit. Rev. Ther. Drug Carrier Systems*, 1991, *8*, 91. It has been found that the uptake and delivery of oligonucleotides can be greatly improved even when administered by non-parenteral means through the use of a number of different classes of penetration enhancers.

In some embodiments, compositions for non-parenteral administration include one or more modifications from naturally-occurring oligonucleotides (i.e. full-phosphodiester deoxyribosyl or full-phosphodiester ribosyl oligonucleotides). Such modifications may increase binding affinity, nuclease stability, cell or tissue permeability, tissue distribution, or other biological or pharmacokinetic property. Modifications may be made to the base, the linker, or the sugar, in general, as discussed in more detail herein with regards to oligonucleotide chemistry. In some embodiments of the invention, compositions for administration to a subject, and in particular oral compositions for administration to an animal or human subject, will comprise modified oligonucleotides having one or more modifications for enhancing affinity, stability, tissue distribution, or other biological property.

Suitable modified linkers include phosphorothioate linkers. In some embodiments according to the invention, the oligomeric compound has at least one phosphorothioate linker. Phosphorothioate linkers provide nuclease stability as well as plasma protein binding characteristics to the compound. Nuclease stability is useful for increasing the *in vivo* lifetime of oligomeric compounds, while plasma protein binding decreases the rate of first pass clearance of oligomeric compound via renal excretion. In some embodiments according to the present invention, the oligomeric compound has at least two phosphorothioate linkers. In some embodiments, wherein the oligomeric compound has exactly n nucleosides, the oligomeric compound has from one to n-1 phosphorothioate linkages. In some embodiments, wherein the oligomeric compound has exactly n nucleosides, the oligomeric compound has n-1 phosphorothioate linkages. In other embodiments wherein the oligomeric compound has exactly n nucleoside, and n is even, the oligomeric compound has from 1 to n/2 phosphorothioate

linkages, or, when n is odd, from 1 to (n-1)/2 phosphorothioate linkages. In some embodiments, the oligomeric compound has alternating phosphodiester (PO) and phosphorothioate (PS) linkages. In other embodiments, the oligomeric compound has at least one stretch of two or more consecutive PO linkages and at least one stretch of two or more PS linkages. In other embodiments, the oligomeric compound has at least two stretches of PO linkages interrupted by at least one PS linkage.

In some embodiments, at least one of the nucleosides is modified on the ribosyl sugar unit by a modification that imparts nuclease stability, binding affinity or some other beneficial biological property to the sugar. In some cases, the sugar modification includes a 2'-modification, e.g. the 2'-OH of the ribosyl sugar is replaced or substituted. Suitable replacements for 2'-OH include 2'-F and 2'-arabino-F. Suitable substitutions for OH include 2'-*O*-alkyl, e.g. 2'-*O*-methyl, and 2'-*O*-substituted alkyl, e.g. 2'-*O*-methoxyethyl, 2'-*O*-aminopropyl, etc. In some embodiments, the oligomeric compound contains at least one 2'-modification. In some embodiments, the oligomeric compound contains at least 2 2'-modifications. In some embodiments, the oligomeric compound has at least one 2'-modification at each of the termini (i.e. the 3'- and 5'-terminal nucleosides each have the same or different 2'-modifications). In some embodiments, the oligomeric compound has at least two sequential 2'-modifications at each end of the compound. In some embodiments, oligomeric compounds further comprise at least one deoxynucleoside. In particular embodiments, oligomeric compounds comprise a stretch of deoxynucleosides such that the stretch is capable of activating RNase (e.g. RNase H) cleavage of an RNA to which the oligomeric compound is capable of hybridizing. In some embodiments, a stretch of deoxynucleosides capable of activating RNase-mediated cleavage of RNA comprises about 8 to about 16, e.g. about 8 to about 16 consecutive deoxynucleosides. In further embodiments, oligomeric compounds are capable of eliciting cleaveage by dsRNAse enzymes.

Oral compositions for administration of non-parenteral oligomeric compounds and compositions of the present invention may be formulated in various dosage forms such as, but not limited to, tablets, capsules, liquid syrups, soft gels, suppositories, and enemas. The term "alimentary delivery" encompasses e.g. oral, rectal, endoscopic and sublingual/buccal administration. A common requirement for these modes of administration is absorption over some portion or all of the alimentary tract and a need for efficient mucosal penetration of the nucleic acid(s) so administered.

Delivery of a drug via the oral mucosa, as in the case of buccal and sublingual administration, has several desirable features, including, in many instances, a more rapid rise in

plasma concentration of the drug than via oral delivery (Harvey, Chapter 35 *In: Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990, page 711).

Endoscopy may be used for delivery directly to an interior portion of the alimentary tract. For example, endoscopic retrograde cystopancreatography (ERCP) takes advantage of extended gastroscopy and permits selective access to the biliary tract and the pancreatic duct (Hirahata *et al.*, *Gan To Kagaku Ryoho*, 1992, *19(10 Suppl.)*, 1591). Pharmaceutical compositions, including liposomal formulations, can be delivered directly into portions of the alimentary canal, such as, *e.g.*, the duodenum (Somogyi *et al.*, *Pharm. Res.*, 1995, *12*, 149) or the gastric submucosa (Akamo *et al.*, *Japanese J. Cancer Res.*, 1994, *85*, 652) via endoscopic means. Gastric lavage devices (Inoue *et al.*, *Artif. Organs*, 1997, *21*, 28) and percutaneous endoscopic feeding devices (Pennington *et al.*, *Ailment Pharmacol. Ther.*, 1995, *9*, 471) can also be used for direct alimentary delivery of pharmaceutical compositions.

In some embodiments, oligomeric compound formulations may be administered through the anus into the rectum or lower intestine. Rectal suppositories, retention enemas or rectal catheters can be used for this purpose and may be preferred when patient compliance might otherwise be difficult to achieve (*e.g.*, in pediatric and geriatric applications, or when the patient is vomiting or unconscious). Rectal administration can result in more prompt and higher blood levels than the oral route. (Harvey, Chapter 35 *In: Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990, page 711). Because about 50% of the drug that is absorbed from the rectum will bypass the liver, administration by this route significantly reduces the potential for first-pass metabolism (Benet *et al.*, Chapter 1 *In: Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th Ed., Hardman *et al.*, eds., McGraw-Hill, New York, NY, 1996).

Some embodiments of the present invention employ various penetration enhancers in order to effect transport of oligomeric compounds and compositions across mucosal and epithelial membranes. Penetration enhancers may be classified as belonging to one of five broad categories - surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p. 92). Penetration enhancers and their uses are described in US Patent 6,287,860, which is incorporated herein in its entirety. Accordingly, some embodiments comprise oral oligomeric compound compositions comprising at least one member of the group consisting of surfactants, fatty acids, bile salts, chelating agents, and non-chelating surfactants. Further embodiments comprise oral oligomeric compound comprising at least one fatty acid, e.g. capric or lauric acid, or combinations or salts

thereof. Other embodiments comprise methods of enhancing the oral bioavailability of an oligomeric compound, the method comprising co-administering the oligomeric compound and at least one penetration enhancer.

Other excipients that may be added to oral oligomeric compound compositions include surfactants (or "surface-active agents"), which are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of oligomeric compounds through the alimentary mucosa and other epithelial membranes is enhanced. In addition to bile salts and fatty acids, surfactants include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether (Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92); and perfluorohemical emulsions, such as FC-43 (Takahashi *et al.*, *J. Pharm. Phamacol.*, 1988, *40,* 252).

Fatty acids and their derivatives which act as penetration enhancers and may be used in compositions of the present invention include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-*rac*-glycerol), dilaurin, caprylic acid, arachidonic acid, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines and mono- and di-glycerides thereof and/or physiologically acceptable salts thereof (*i.e.*, oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, *etc.*) (Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, *7,* 1; El-Hariri *et al.*, *J. Pharm. Pharmacol.*, 1992, *44,* 651).

In some embodiments, oligomeric compound compositions for oral delivery comprise at least two discrete phases, which phases may comprise particles, capsules, gel-capsules, microspheres, etc. Each phase may contain one or more oligomeric compounds, penetration enhancers, surfactants, bioadhesives, effervescent agents, or other adjuvant, excipient or diluent. In some embodiments, one phase comprises at least one oligomeric compound and at least one penetration enhancer. In some embodiments, a first phase comprises at least one oligomeric compound and at least one penetration enhancer, while a second phase comprises at least one penetration enhancer. In some embodiments, a first phase comprises at least one oligomeric compound and at least one penetration enhancer, while a second phase comprises at least one penetration enhancer and substantially no oligomeric compound. In some embodiments, at least one phase is compounded with at least one degradation retardant, such as a coating or a matrix, which delays release of the contents of that phase. In some embodiments, a first phase comprises at least one oligomeric compound, at least one penetration enhancer, while a second

phase comprises at least one penetration enhancer and a release-retardant. In particular embodiments, an oral oligomeric compound comprises a first phase comprising particles containing an oligomeric compound and a penetration enhancer, and a second phase comprising particles coated with a release-retarding agent and containing penetration enhancer.

A variety of bile salts also function as penetration enhancers to facilitate the uptake and bioavailability of drugs. The physiological roles of bile include the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 *In: Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th Ed., Hardman *et al.*, eds., McGraw-Hill, New York, NY, 1996, pages 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus, the term "bile salt" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. The bile salts of the invention include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (CDCA, sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92; Swinyard, Chapter 39 *In: Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990, pages 782-783; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, *7*, 1; Yamamoto *et al.*, *J. Pharm. Exp. Ther.*, 1992, *263*, 25; Yamashita *et al.*, *J. Pharm. Sci.*, 1990, *79*, 579).

In some embodiments, penetration enhancers useful in some embodiments of present invention are mixtures of penetration enhancing compounds. One such penetration enhancer is a mixture of UDCA (and/or CDCA) with capric and/or lauric acids or salts thereof e.g. sodium. Such mixtures are useful for enhancing the delivery of biologically active substances across mucosal membranes, in particular intestinal mucosa. Other penetration enhancer mixtures comprise about 5-95% of bile acid or salt(s) UDCA and/or CDCA with 5-95% capric and/or lauric acid. Particular penetration enhancers are mixtures of the sodium salts of UDCA, capric acid and lauric acid in a ratio of about 1:2:2 respectively. Anther such penetration enhancer is a mixture of capric and lauric acid (or salts thereof) in a 0.01:1 to 1:0.01 ratio (mole basis). In particular embodiments capric acid and lauric acid are present in molar ratios of e.g. about 0.1:1 to about 1:0.1, in particular about 0.5:1 to about 1:0.5.

Other excipients include chelating agents, i.e. compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of oligomeric compounds through the alimentary and other mucosa is enhanced. With regard to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, *J. Chromatogr.*, 1993, *618*, 315). Chelating agents of the invention include, but are not limited to, disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (*e.g.*, sodium salicylate, 5-methoxysalicylate and homovanilate), *N*-acyl derivatives of collagen, laureth-9 and *N*-amino acyl derivatives of beta-diketones (enamines)(Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, *7*, 1; Buur *et al.*, *J. Control Rel.*, 1990, *14*, 43).

As used herein, non-chelating non-surfactant penetration enhancers may be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of oligomeric compounds through the alimentary and other mucosal membranes (Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, *7*, 1). This class of penetration enhancers includes, but is not limited to, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita *et al.*, *J. Pharm. Pharmacol.*, 1987, *39*, 621).

Agents that enhance uptake of oligomeric compounds at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi *et al*, U.S. Patent No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo *et al.*, PCT Application WO 97/30731), can be used.

Some oral oligomeric compound compositions also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which may be inert (*i.e.*, does not possess biological activity *per se*) or may be necessary for transport, recognition or pathway activation or mediation, or is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of an oligomeric compound having biological activity by, for example, degrading the biologically active oligomeric compound or promoting its removal from circulation. The coadministration of a oligomeric compound and a carrier compound, typically with an excess of the latter substance, can result in

a substantial reduction of the amount of oligomeric compound recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the oligomeric compound for a common receptor. For example, the recovery of a partially phosphorothioate oligomeric compound in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao *et al.*, *Antisense Res. Dev.*, 1995, *5,* 115; Takakura *et al.*, *Antisense & Nucl. Acid Drug Dev.*, 1996, *6,* 177).

A "pharmaceutical carrier" or "excipient" may be a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more oligomeric compounds to an animal. The excipient may be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, *etc.*, when combined with an oligomeric compound and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, *etc.*); fillers (*e.g.*, lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, *etc.*); lubricants (*e.g.*, magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, *etc.*); disintegrants (*e.g.*, starch, sodium starch glycolate, EXPLOTAB); and wetting agents (*e.g.*, sodium lauryl sulphate, *etc.*).

Oral oligomeric compound compositions may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipuritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the composition of present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention.

The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active

ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The oligomeric compounds and compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, foams and liposome-containing formulations.

Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. Emulsions may contain additional components in addition to the dispersed phases, and the active drug that may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Microemulsions are included as an embodiment of the present invention. Emulsions and their uses are well known in the art and are described in U.S. Patent 6,287,860, which is incorporated herein in its entirety.

Formulations of the present invention include liposomal formulations. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes which are believed to interact with negatively charged nucleic acid molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap nucleic acids rather than complex with it. Both cationic and noncationic liposomes have been used to deliver nucleic acids and oligomeric compounds to cells.

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. Liposomes and their uses are described in U.S. Patent 6,287,860, which is incorporated herein in its entirety.

The pharmaceutical formulations and compositions of the present invention may also include surfactants. The use of surfactants in drug products, formulations and in emulsions is well known in the art. Surfactants and their uses are described in U.S. Patent 6,287,860, which is incorporated herein in its entirety.

One of skill in the art will recognize that formulations are routinely designed according to their intended use, i.e. route of administration.

Formulations for topical administration include those in which the oligomeric compounds of the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Lipids and liposomes include neutral (e.g. dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl choline) negative (e.g. dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g. dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA).

For topical or other administration, oligomeric compounds and compositions of the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, they may be complexed to lipids, in particular to cationic lipids. Topical formulations are described in detail in U.S. patent application 09/315,298 filed on May 20, 1999, which is incorporated herein by reference in its entirety.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Oral formulations are those in which oligomeric compounds of the invention are administered in conjunction with one or more penetration enhancers surfactants and chelators. A particularly suitable combination is the sodium salt of lauric acid, capric acid and UDCA. Penetration enhancers also include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. Compounds and compositions of the invention may be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. Certain oral formulations for oligonucleotides and their preparation are described in detail in U.S. applications 09/108,673 (filed July 1, 1998), 09/315,298 (filed May 20, 1999) and U.S. Application Publication 20030027780, each of which is incorporated herein by reference in their entirety.

Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions that may also contain buffers, diluents and

other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Certain embodiments of the invention provide pharmaceutical compositions containing one or more of the compounds and compositions of the invention and one or more other chemotherapeutic agents that function by a non-antisense mechanism. Examples of such chemotherapeutic agents include but are not limited to cancer chemotherapeutic drugs such as daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES). When used with the oligomeric compounds of the invention, such chemotherapeutic agents may be used individually (*e.g.*, 5-FU and oligonucleotide), sequentially (*e.g.*, 5-FU and oligonucleotide for a period of time followed by MTX and oligonucleotide), or in combination with one or more other such chemotherapeutic agents (*e.g.*, 5-FU, MTX and oligonucleotide, or 5-FU, radiotherapy and oligonucleotide). Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. Combinations of oligomeric compounds and compositions of the invention and other drugs are also within the scope of this invention. Two or more combined compounds such as two oligomeric compounds or one oligomeric compound combined with further compounds may be used together or sequentially.

In another embodiment, compositions of the invention may contain one or more of the compounds and compositions of the invention targeted to a first nucleic acid target and one or more additional oligomeric compounds targeted to a second nucleic acid target. Alternatively, compositions of the invention may contain two or more oligomeric compounds and compositions targeted to different regions, segments or sites of the same target. Two or more combined compounds may be used together or sequentially.

The formulation of therapeutic compounds and compositions of the invention and their subsequent administration (dosing) is believed to be within the skill of those in the art. Dosing is

dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligomeric compounds, and can generally be estimated based on EC_{50}s found to be effective in *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 μg to 100 g per kg of body weight, from 0.1 μg to 10 g per kg of body weight, from 1.0 μg to 1 g per kg of body weight, from 10.0 μg to 100 mg per kg of body weight, from 100 μg to 10 mg per kg of body weight, or from 1 mg to 5 mg per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily determine repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligomeric compound is administered in maintenance doses, ranging from 0.01 μg to 100 g per kg of body weight, from 0.1 μg to 10 g per kg of body weight, from 1 μg to 1 g per kg of body weight, from 10 μg to 100 mg per kg of body weight, from 100 μg to 10 mg per kg of body weight, or from 100 μg to 1 mg per kg of body weight, once or more daily, to once every 20 years. The effects of treatments with therapeutic compositions can be assessed following collection of tissues or fluids from a patient or subject receiving said treatments. It is known in the art that a biopsy sample can be procured from certain tissues without resulting in detrimental effects to a patient or subject. In certain embodiments, a tissue and its constituent cells comprise, but are not limited to, blood (e.g., hematopoietic cells, such as human hematopoietic progenitor cells, human hematopoietic stem cells, $CD34^+$ cells $CD4^+$ cells), lymphocytes and other blood lineage cells, bone marrow, breast, cervix, colon, esophagus, lymph node, muscle, peripheral blood, oral mucosa and skin. In other embodiments, a fluid and its constituent cells comprise, but are not limited to, blood, urine, semen, synovial fluid, lymphatic fluid and cerebro-spinal fluid. Tissues or fluids procured from patients can be evaluated for expression levels of a target small non-coding RNA, mRNA or protein. Additionally, the mRNA or protein expression levels of other genes known or suspected to be associated with the specific disease state, condition or phenotype can be assessed. mRNA levels can be measured or evaluated by real-time PCR, Northern blot, in situ hybridization or DNA array analysis.

Protein levels of a downstream target modulated or regulated by a small non-coding RNA can be evaluated or quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art.

Western blot analysis of protein levels:

When small non-coding RNAs have effects on expression of downstream genes or proteins encoded by genes, it is advantageous to measure the protein levels of those gene products. To do this, western blot analysis may be employed.

Western blot analysis (immunoblot analysis) is carried out using standard methods. Cells are harvested 16-20 h after oligomeric compound treatment, washed once with PBS, suspended in Laemmli buffer (100 µl/well), boiled for 5 minutes and loaded on a 16% SDS-PAGE gel. Gradient gels (4-20%) may also be used for the separation of proteins, as is known in the art. Gels are typically run for 1.5 hours at 150 V, and transferred to a membrane, such as PVDF, for western blotting. Appropriate primary antibody directed to a target is used, with a radiolabeled or fluorescently labeled secondary antibody directed against the primary antibody species. Bands are visualized using a PHOSPHORIMAGER™ (Molecular Dynamics, Sunnyvale CA).

Furthermore, the effects of treatment can be assessed by measuring biomarkers associated with the disease or condition in the aforementioned tissues and fluids, collected from a patient or subject receiving treatment, by routine clinical methods known in the art. These biomarkers include but are not limited to: glucose, cholesterol, lipoproteins, triglycerides, free fatty acids and other markers of glucose and lipid metabolism; liver transaminases, bilirubin, albumin, blood urea nitrogen, creatine and other markers of kidney and liver function; interleukins, tumor necrosis factors, intracellular adhesion molecules, C-reactive protein and other markers of inflammation; testosterone, estrogen and other hormones; tumor markers; vitamins, minerals and electrolytes.

In vitro and *in vivo* assays:

Phenotypic assays

Once modulators are designed or identified by the methods disclosed herein, the oligomeric compounds are further investigated in one or more phenotypic assays, each having measurable endpoints predictive or suggestive of efficacy in the treatment, amelioration or improvement of physiologic conditions associated with a particular disease state or condition.

Phenotypic assays, kits and reagents for their use are well known to those skilled in the art and are herein used to investigate the role and/or association of a target in health and disease. Representative phenotypic assays, which can be purchased from any one of several commercial vendors, include those for determining cell viability, cytotoxicity, proliferation or cell survival (Molecular Probes, Eugene, OR; PerkinElmer, Boston, MA), protein-based assays including enzymatic assays (Panvera, LLC, Madison, WI; BD Biosciences, Franklin Lakes, NJ; Oncogene Research Products, San Diego, CA), cell regulation, signal transduction, inflammation, oxidative processes and apoptosis (Assay Designs Inc., Ann Arbor, MI), triglyceride accumulation (Sigma-Aldrich, St. Louis, MO), angiogenesis assays, tube formation assays, cytokine and hormone assays and metabolic assays (Chemicon International Inc., Temecula, CA; Amersham Biosciences, Piscataway, NJ).

In one non-limiting example, cells determined to be appropriate for a particular phenotypic assay (i.e., MCF7 cells selected for breast cancer studies; adipocytes for obesity studies) are treated with an oligomeric compound identified from the *in vitro* studies as well as control compounds at optimal concentrations which are determined by the methods described above. At the end of the treatment period, treated and untreated cells are analyzed by one or more methods specific for the assay to determine phenotypic outcomes and endpoints.

Phenotypic endpoints include changes in cell morphology over time or treatment dose as well as changes in levels of cellular components such as proteins, lipids, nucleic acids, hormones, saccharides or metals. Measurements of cellular status which include pH, stage of the cell cycle, intake or excretion of biological indicators by the cell, are also endpoints of interest.

Analysis of the genotype of the cell (measurement of the expression of one or more of the genes of the cell) after treatment is also used as an indicator of the efficacy or potency of the oligomeric compound. Hallmark genes, or those genes suspected to be associated with a specific disease state, condition, or phenotype, are measured in both treated and untreated cells.

Cell proliferation and survival assays:

In some embodiments, cell proliferation and survival assays are used. Cell cycle regulation is the basis for many cancer therapeutic agents. Unregulated cell proliferation is a characteristic of cancer cells, thus most current chemotherapy agents target dividing cells, for example, by blocking the synthesis of new DNA required for cell division. However, cells in

healthy tissues are often also affected by agents that modulate cell proliferation.

In some cases, a cell cycle inhibitor will cause apoptosis in cancer cells, but allow normal cells to undergo growth arrest and therefore remain unaffected (Blagosklonny, *Bioessays,* **1999**, *21*, 704-709; Chen et al., *Cancer Res.,* **1997**, *57*, 2013-2019; Evan and Littlewood, *Science,* **1998**, *281*, 1317-1322; Lees and Weinberg, *Proc. Natl. Acad. Sci. USA,* **1999**, *96*, 4221-4223). An example of sensitization to anti-cancer agents is observed in cells that have reduced or absent expression of the tumor suppressor genes p53 (Bunz et al., *Science,* **1998**, *282*, 1497-1501; Bunz et al., *J. Clin. Invest.,* **1999**, *104*, 263-269; Stewart et al., *Cancer Res.,* **1999**, *59*, 3831-3837; Wahl et al., *Nat. Med.,* **1996**, *2*, 72-79). However, cancer cells often escape apoptosis (Lowe and Lin, *Carcinogenesis,* **2000**, *21*, 485-495; Reed, *Cancer J. Sci. Am.,* **1998**, *4 Suppl 1*, S8-14). Further disruption of cell cycle checkpoints in cancer cells can increase sensitivity to chemotherapy while allowing normal cells to take refuge in G1 and remain unaffected.

Cell cycle assay:

A cell cycle assay is employed to identify genes whose modulation affects cell cycle progression. In addition to normal cells, cells lacking functional p53 are utilized to identify genes whose modulation will sensitize p53-deficient cells to anti-cancer agents. Oligomeric compounds of the invention are tested for their effects on the cell cycle in normal human mammary epithelial cells (HMECs) as well as the breast carcinoma cell lines MCF7 and T47D. The latter two cell lines express similar genes but MCF7 cells express the tumor suppressor p53, while T47D cells are deficient in p53. A 20-nucleotide oligomeric compound with a randomized sequence may be used as a negative control, as it does not target modulators of cell cycle progression. An oligomeric compound targeting kinesin-like 1 is known to inhibit cell cycle progression and may be used as a positive control.

Cells are transfected as described herein. Oligomeric compounds are mixed with LIPOFECTIN™ in OPTI-MEM™ to achieve a final concentration of 200 nM of oligomeric compound and 6 μg/mL LIPOFECTIN™. Compounds of the invention and the positive control are tested in triplicate. The negative control is tested in up to six replicate wells. Untreated control cells receive LIPOFECTIN™ only. Approximately 24, 48 or 72 hours following transfection, routine procedures are used to prepare cells for flow cytometry analysis and cells are stained with propidium iodide to generate a cell cycle profile using a flow cytometer. The cell cycle profile is analyzed with the ModFit program (Verity Software House, Inc., Topsham ME).

Fragmentation of nuclear DNA is a hallmark of apoptosis and produces an increase in cells with a hypodiploid DNA content, which are categorized as "subG1." An increase in cells

in G1 phase is indicative of a cell cycle arrest prior to entry into S phase; an increase in cells in S phase is indicative of cell cycle arrest during DNA synthesis; and an increase in cells in the G2/M phase is indicative of cell cycle arrest just prior to or during mitosis. Cell cycle profiles of cells treated with oligomeric compounds can be normalized to those of untreated control cells, and values above or below 100% are considered to indicate an increase or decrease, respectively, in the proportion of cells in a particular phase of the cell cycle.

Oligomeric compounds that prevent cell cycle progression are candidate therapeutic agents for the treatment of hyperproliferative disorders, such as cancer or inflammation.

Caspase Assay:

Programmed cell death, or apoptosis, is an important aspect of various biological processes, including normal cell turnover, immune system development and embryonic development. Apoptosis involves the activation of caspases, a family of intracellular proteases through which a cascade of events leads to the cleavage of a select set of proteins. The caspase family can be divided into two groups: the initiator caspases, such as caspase-8 and –9, and the executioner caspases, such as caspase-3, -6 and –7, which are activated by the initiator caspases. The caspase family contains at least 14 members, with differing substrate preferences (Thornberry and Lazebnik, *Science,* **1998**, *281*, 1312-1316). A caspase assay is utilized to identify genes whose modulation causes apoptosis. The chemotherapeutic drugs taxol, cisplatin, etoposide, gemcitabine, camptothecin, aphidicolin and 5-fluorouracil all have been shown to induce apoptosis in a caspase-dependent manner.

In a further embodiment, a caspase assay is employed to identify genes or targets whose modulation affects apoptosis. In addition to normal cells, cells lacking functional p53 are utilized to identify genes or targets whose modulation will sensitize p53-deficient cells to agents that induce apoptosis. Oligomeric compounds of the invention are assayed for their affects on apoptosis in normal HMECs as well as the breast carcinoma cell lines MCF7 and T47D. HMECs and MCF7 cells express p53, whereas T47D cells do not express this tumor suppressor gene. Cells are cultured in 96-well plates with black sides and flat, transparent bottoms (Corning Incorporated, Corning, NY). DMEM medium, with and without phenol red, is obtained from Invitrogen Life Technologies (Carlsbad, CA). MEGM medium, with and without phenol red, is obtained from Cambrex Bioscience (Walkersville, MD). A 20-nucleotide oligomeric compound with a randomized sequence may be used as a negative control, as it does not target modulators of caspase activity. An oligomeric compound targeted to human Jagged2 or human Notch1, both of which are known to induce caspase activity, may be used as a positive control for caspase activation.

Cells are transfected as described herein. Oligomeric componds are mixed with LIPOFECTIN™ in OPTI-MEM™ to achieve a final concentration of 200 nM of oligomeric compound and 6 μg/mL LIPOFECTIN™. Compounds of the invention and the positive controls are tested in triplicate, and the negative control is tested in up to six replicate wells. Untreated control cells receive LIPOFECTIN™ only.

Caspase-3 activity is evaluated with a fluorometric HTS Caspase-3 assay (Catalog # HTS02; EMD Biosciences, San Diego, CA) that detects cleavage after aspartate residues in the peptide sequence DEVD. The DEVD substrate is labeled with a fluorescent molecule, which exhibits a blue to green shift in fluorescence upon cleavage by caspase-3. Active caspase-3 in the oligomeric compound-treated cells is measured by this assay according to the manufacturer's instructions. Approximately 48 hours following treatment, 50 μL of assay buffer containing 10 μM dithiothreitol is added to each well, followed by addition 20 μL of the caspase-3 fluorescent substrate conjugate. Fluorescence in wells is immediately detected (excitation/emission 400/505 nm) using a fluorescent plate reader (SpectraMAX GeminiXS, Molecular Devices, Sunnyvale, CA). The plate is covered and incubated at 37°C for an additional three hours, after which the fluorescence is again measured (excitation/emission 400/505 nm). The value at time zero is subtracted from the measurement obtained at 3 hours. The measurement obtained from the untreated control cells is designated as 100% activity. Caspase-3 activity in cells treated with oligomeric compounds is normalized to that in untreated control cells. Values for caspase activity above or below 100% are considered to indicate that the compound has the ability to stimulate or inhibit caspase activity, respectively.

Oligomeric compounds that cause a significant induction in apoptosis are candidate therapeutic agents with applications in the treatment of conditions in which the induction of apoptosis is desirable, for example, in hyperproliferative disorders. Oligomeric compounds that inhibit apoptosis are candidate therapeutic agents with applications in the treatment of conditions where the reduction of apoptosis is useful, for example, in neurodegenerative disorders.

Angiogenesis assays:

In some embodiments, angiogenesis assays are used. Angiogenesis is the growth of new blood vessels (veins and arteries) by endothelial cells. This process is important in the development of a number of human diseases, and is believed to be particularly important in regulating the growth of solid tumors. Without new vessel formation it is believed that tumors will not grow beyond a few millimeters in size. In addition to their use as anti-cancer agents, inhibitors of angiogenesis have potential for the treatment of diabetic retinopathy, cardiovascular disease, rheumatoid arthritis and psoriasis (Carmeliet and Jain, *Nature,* **2000**, *407*, 249-257;

Freedman and Isner, *J. Mol. Cell. Cardiol.*, **2001**, *33*, 379-393; Jackson et al., *Faseb J.*, **1997**, *11*, 457-465; Saaristo et al., *Oncogene,* **2000**, *19*, 6122-6129; Weber and De Bandt, *Joint Bone Spine,* **2000**, *67*, 366-383; Yoshida et al., *Histol. Histopathol.*, **1999**, *14*, 1287-1294).

Expression of angiogenic genes as a measure of angiogenesis:

During the process of angiogenesis, endothelial cells perform several distinct functions, including the degradation of the extracellular matrix (ECM), migration, proliferation and the formation of tube-like structures (Liekens et al., *Biochem. Pharmacol.*, **2001**, *61*, 253-270). Endothelial cells must regulate the expression of many genes in order to perform the functions necessary for angiogenesis. This gene regulation has been the subject of intense scrutiny, and many genes have been identified as being important for the angiogenic phenotype. Genes highly expressed in angiogenic endothelial cells include integrin β3, endoglin/CD105, TEM5 and MMP-14/MT-MMP1.

Integrin β3 is part of a family of heterodimeric transmembrane receptors that consist of alpha and beta subunits (Brooks et al., *J. Clin. Invest.*, **1995**, *96*, 1815-1822). Each subunit recognizes a unique set of ECM ligands, thereby allowing cells to transmit angiogenic signals from the extracellular matrix. Integrin β3 is prominently expressed on proliferating vascular endothelial cells, and it plays roles in allowing new blood vessels to form at tumor sites as well as allowing the epithelial cells of breast tumors to spread (Brooks et al., *J. Clin. Invest.*, **1995**, *96*, 1815-1822; Drake et al., *J. Cell Sci.*, **1995**, *108 (Pt 7)*, 2655-2661). Blockage of integrin β3 with monoclonal antibodies or low molecular weight antagonists inhibits blood vessel formation in a variety of in-vivo models, including tumor angiogenesis and neovascularization during oxygen-induced retinopathy (Brooks et al., *Science,* **1994**, *264*, 569-571; Brooks et al., *J. Clin. Invest.*, **1995**, *96*, 1815-1822; Hammes et al., *Nat. Med.*, **1996**, *2*, 529-533).

Endoglin is a transforming growth factor receptor-associated protein highly expressed on endothelial cells, and present on some leukemia cells and minor subsets of bone marrow cells (Burrows et al., *Clin. Cancer Res.*, **1995**, *1*, 1623-1634; Haruta and Seon, *Proc. Natl. Acad. Sci. U S A,* **1986**, *83*, 7898-7902). Its expression is upregulated in endothelial cells of angiogenic tissues and is therefore used as a prognostic indicator in various tumors (Burrows et al., *Clin. Cancer Res.*, **1995**, *1*, 1623-1634). Endoglin functions as an ancillary receptor influencing binding of the transforming growth factor beta (TGF-beta) family of ligands to signaling receptors, thus mediating cell survival (Massague and Chen, *Genes Dev.*, **2000**, *14*, 627-644).

Tumor endothelial marker 5 (TEM5) is a putative 7-pass transmembrane protein (GPCR) (Carson-Walter et al., *Cancer Res.*, **2001**, *61*, 6649-6655). The mRNA transcript, designated KIAA1531, encodes one of many tumor endothelium markers (TEMs) that display

elevated expression (greater than 10-fold) during tumor angiogenesis (St Croix et al., *Science,* **2000**, *289*, 1197-1202). TEM5 is coordinately expressed with other TEMs on tumor endothelium in humans and mice.

Matrix metalloproteinase 14 (MMP-14), a membrane-type MMP covalently linked to the cell membrane, is involved in matrix detachment and migration. MMP-14 is thought to promote tumor angiogenesis; antibodies directed against the catalytic domain of MMP-14 block endothelial-cell migration, invasion and capillary tube formation *in vitro* (Galvez et al., *J. Biol. Chem.*, **2001**, *276*, 37491-37500). MMP-14 can degrade the fibrin matrix that surrounds newly formed vessels potentially allowing the endothelial cells to invade further into the tumor tissue (Hotary et al., *J. Exp. Med.*, **2002**, *195*, 295-308). MMP-14 null mice have impaired angiogenesis during development, further demonstrating the role of MMP-14 in angiogenesis (Vu and Werb, *Genes Dev.*, **2000**, *14*, 2123-2133; Zhou et al., *Proc. Natl. Acad. Sci. USA,* **2000**, *97*, 4052-4057).

In some embodiments, HUVECs are used to measure the effects of oligomeric compounds of the invention on the activity of endothelial cells stimulated with human vascular endothelial growth factor (VEGF). A 20-nucleotide oligomeric compound with a randomized sequence may be used as a negative control, as it does not target modulators of HUVEC activity.

Cells are transfected as described herein. Oligomeric componds are mixed with LIPOFECTIN™ in OPTI-MEM™ to achieve a final concentration of 75 nM of oligomeric compound and 2.25 μg/mL LIPOFECTIN™. Compounds of the invention are tested in triplicate, and the negative control is tested in up to six replicate wells. Untreated control cells receive LIPOFECTIN™ only.

Approximately twenty hours after transfection, cells are induced to express angiogenic genes with recombinant VEGF. Total RNA is harvested approximately 52 hours following transfection, and the amount of total RNA from each sample is determined using a Ribogreen Assay (Invitrogen Life Technologies, Carlsbad, CA). Real-time RT-PCR is performed on the total RNA using primer/probe sets for four angiogenic hallmark genes described herein: integrin β3, endoglin, TEM5 and MMP14. Expression levels for each gene are normalized to total RNA. Gene expression in cells treated with oligomeric compounds is normalized to that in untreated control cells. A value above or below 100% is considered to indicated an increase or decrease in gene expression, respectively.

Oligomeric compounds resulting in a decrease in the expression of angiogenic hallmark genes are candidate therapeutic agents for the inhibition of angiogenesis where such activity is desired, for example, in the treatment of cancer, diabetic retinopathy, cardiovascular disease,

rheumatoid arthritis and psoriasis. Oligomeric compounds that increase the expression of angiogenic hallmark genes are candidate therapeutic agents with applications where the stimulation of angiogenesis is desired, for example, in wound healing.

Endothelial tube formation assay as a measure of angiogenesis:

Angiogenesis is stimulated by numerous factors that promote interaction of endothelial cells with each other and with extracellular matrix molecules, resulting in the formation of capillary tubes. This morphogenic process is necessary for the delivery of oxygen to nearby tissues and plays an essential role in embryonic development, wound healing, and tumor growth (Carmeliet and Jain, *Nature,* **2000**, *407*, 249-257). Moreover, this process can be reproduced in a tissue culture assay that evaluated the formation of tube-like structures by endothelial cells. There are several different variations of the assay that use different matrices, such as collagen I (Kanayasu et al., *Lipids,* **1991**, *26*, 271-276), Matrigel (Yamagishi et al., *J. Biol. Chem.,* **1997**, *272*, 8723-8730) and fibrin (Bach et al., *Exp. Cell Res.,* **1998**, *238*, 324-334), as growth substrates for the cells. In this assay, HUVECs are plated on a matrix derived from the Engelbreth-Holm-Swarm mouse tumor, which is very similar to Matrigel (Kleinman et al., *Biochemistry,* **1986**, *25*, 312-318; Madri and Pratt, *J. Histochem. Cytochem.,* **1986**, *34*, 85-91). Untreated HUVECs form tube-like structures when grown on this substrate. Loss of tube formation in vitro has been correlated with the inhibition of angiogenesis in vivo (Carmeliet and Jain, *Nature,* **2000**, *407*, 249-257; Zhang et al., *Cancer Res.,* **2002**, *62*, 2034-2042), which supports the use of in vitro tube formation as an endpoint for angiogenesis.

In some embodiments, HUVECs are used to measure the effects of oligomeric compounds of the invention on endothelial tube formation activity. The tube formation assay is performed using an *in vitro* Angiogenesis Assay Kit (Chemicon International, Temecula, CA). A 20-nucleotide oligomeric compound with a randomized sequence may be used as a negative control, as it does not target modulators of endothelial tube formation.

Oligomeric compounds are mixed with LIPOFECTIN™ in OPTI-MEM™ to achieve a final concentration of 75 nM of oligomeric compound and 2.25 μg/mL LIPOFECTIN™. Untreated control cells receive LIPOFECTIN™ only. Compounds of the invention are tested in triplicate, and the negative control is tested in up to six replicates.

Approximately fifty hours after transfection, cells are transferred to 96-well plates coated with ECMatrix™ (Chemicon International). Under these conditions, untreated HUVECs form tube-like structures. After an overnight incubation at 37° C, treated and untreated cells are inspected by light microscopy. Tube formation in cells treated with oligomeric compounds is compared to that in untreated control cells. Individual wells are assigned discrete scores from 1

to 5 depending on the extent of tube formation. A score of 1 refers to a well with no tube formation while a score of 5 is given to wells where all cells are forming an extensive tubular network.

Oligomeric compounds resulting in a decrease in tube formation are candidate therapeutic agents for the inhibition of angiogenesis where such activity is desired, for example, in the treatment of cancer, diabetic retinopathy, cardiovascular disease, rheumatoid arthritis and psoriasis. Oligomeric compounds that promote endothelial tube formation are candidate therapeutic agents with applications where the stimulation of angiogenesis is desired, for example, in wound healing.

Matrix Metalloproteinase Activity:

During angiogenesis, endothelial cells must degrade the extracellular matrix (ECM) and thus secrete matrix metalloproteinases (MMPs) in order to accomplish this degradation. MMPs are a family of zinc-dependent endopeptidases that fall into eight distinct classes: five are secreted and three are membrane-type MMPs (MT-MMPs) (Egeblad and Werb, *J. Cell Science*, **2002**, *2*, 161-174). MMPs exert their effects by cleaving a diverse group of substrates, which include not only structural components of the extracellular matrix, but also growth-factor-binding proteins, growth-factor pre-cursors, receptor tyrosine-kinases, cell-adhesion molecules and other proteinases (Xu et al., *J. Cell Biol.*, **2002**, *154*, 1069-1080).

In some embodiments, oligomeric compounds of the invention are evaluated for their effects on MMP activity in the medium above cultured HUVECs. MMP activity is measured using the EnzChek Gelatinase/Collagenase Assay Kit (Molecular Probes, Eugene, OR). In this assay, HUVECs are plated at approximately 4000 cells per well in 96-well plates and transfected one day later. A 20-nucleotide oligomeric compound with a randomized sequence may be used as a negative control, as it does not target modulators of MMP activity. An oligomeric compound targeted to integrin β3 is known to inhibit MMP activity and may be used as a positive control.

Cells are transfected as described herein. Oligomeric compounds are mixed with LIPOFECTIN™ in OPTI-MEM™ to achieve a final concentration of 75 nM of oligomeric compound and 2.25 μg/mL LIPOFECTIN™. Compounds of the invention and the positive control are tested in triplicate, and the negative control is tested in up to six replicates. Untreated control cells receive LIPOFECTIN™ only.

Approximately 50 hours after transfection, a p-aminophenylmercuric acetate (APMA, Sigma-Aldrich, St. Louis, MO) solution is added to each well of a Corning-Costar 96-well clear bottom plate (VWR International, Brisbane, CA). The APMA solution is used to promote

cleavage of inactive MMP precursor proteins. Medium above the HUVECs is then transferred to the wells in the 96-well plate. After approximately 30 minutes, the quenched, fluorogenic MMP cleavage substrate is added, and baseline fluorescence is read immediately at 485 nm excitation/530 nm emission. Following an overnight incubation at 37°C in the dark, plates are read again to determine the amount of fluorescence, which corresponds to MMP activity. Total protein from HUVEC lysates is used to normalize the readings, and MMP activity from cells treated with oligomeric compounds is normalized to that of untreated control cells. MMP activities above or below 100% are considered to indicate a stimulation or inhibition, respectively, of MMP activity.

Oligomeric compounds resulting in a decrease in MMP activity are candidate therapeutic agents for the inhibition of angiogenesis where such activity is desired, for example, in the treatment of cancer, diabetic retinopathy, cardiovascular disease, rheumatoid arthritis and psoriasis. Oligomeric compounds that increase the expression of angiogenic hallmark genes are candidate therapeutic agents with applications in conditions requiring angiogenesis, for example, in wound healing.

Adipocyte assays:

In some embodiments, adipocytes assays are used. Insulin is an essential signaling molecule throughout the body, but its major target organs are the liver, skeletal muscle and adipose tissue. Insulin is the primary modulator of glucose homeostasis and helps maintain a balance of peripheral glucose utilization and hepatic glucose production. The reduced ability of normal circulating concentrations of insulin to maintain glucose homeostasis manifests in insulin resistance which is often associated with diabetes, central obesity, hypertension, polycystic ovarian syndrome, dyslipidemia and atherosclerosis (Saltiel, *Cell,* **2001**, *104*, 517-529; Saltiel and Kahn, *Nature,* **2001**, *414*, 799-806).

Response of undifferentiated adipocytes to insulin:

Insulin promotes the differentiation of preadipocytes into adipocytes. The condition of obesity, which results in increases in fat cell number, occurs even in insulin-resistant states in which glucose transport is impaired due to the antilipolytic effect of insulin. Inhibition of triglyceride breakdown requires much lower insulin concentrations than stimulation of glucose transport, resulting in maintenance or expansion of adipose stores (Kitamura et al., *Mol. Cell. Biol.,* **1999**, *19*, 6286-6296; Kitamura et al., *Mol. Cell. Biol.,* **1998**, *18*, 3708-3717).

One of the hallmarks of cellular differentiation is the upregulation of gene expression. During adipocyte differentiation, the gene expression patterns in adipocytes change considerably. Some genes known to be upregulated during adipocyte differentiation include

hormone-sensitive lipase (HSL), adipocyte lipid binding protein (aP2), glucose transporter 4 (Glut4), and peroxisome proliferator-activated receptor gamma (PPAR-γ). Insulin signaling is improved by compounds that bind and inactivate PPAR-γ, a key regulator of adipocyte differentiation (Olefsky, *J. Clin. Invest.*, **2000**, *106*, 467-472). Insulin induces the translocation of GLUT4 to the adipocyte cell surface, where it transports glucose into the cell, an activity necessary for triglyceride synthesis. In all forms of obesity and diabetes, a major factor contributing to the impaired insulin-stimulated glucose transport in adipocytes is the downregulation of GLUT4. Insulin also induces hormone sensitive lipase (HSL), which is the predominant lipase in adipocytes that functions to promote fatty acid synthesis and lipogenesis (Fredrikson et al., *J. Biol. Chem.*, **1981**, *256*, 6311-6320). Adipocyte fatty acid binding protein (aP2) belongs to a multi-gene family of fatty acid and retinoid transport proteins. aP2 is postulated to serve as a lipid shuttle, solubilizing hydrophobic fatty acids and delivering them to the appropriate metabolic system for utilization (Fu et al., *J. Lipid Res.*, **2000**, *41*, 2017-2023; Pelton et al., *Biochem. Biophys. Res. Commun.*, **1999**, *261*, 456-458). Together, these genes play important roles in the uptake of glucose and the metabolism and utilization of fats.

Leptin secretion and an increase in triglyceride content are also well-established markers of adipocyte differentiation. In addition to its role in adipocytes differentiation, leptin also regulates glucose homeostasis through mechanisms (autocrine, paracrine, endocrine and neural) independent of the adipocyte's role in energy storage and release. As adipocytes differentiate, insulin increases triglyceride accumulation by both promoting triglyceride synthesis and inhibiting triglyceride breakdown (Spiegelman and Flier, *Cell*, **2001**, *104*, 531-543). As triglyceride accumulation correlates tightly with cell size and cell number, it is an excellent indicator of differentiated adipocytes.

Oligomeric compounds of the invention are tested for their effects on preadipocyte differentiation. A 20-nucleotide oligomeric compound with a randomized sequence may be used as a negative control, as it does not target modulators of adipocyte differentiation. Tumor necrosis factor alpha (TNF-α) is known to inhibit adipocyte differentiation and may be used as a positive control for the inhibition of adipocyte differentiation as evaluated by leptin secretion. For the other adipocyte differentiation markers assayed, an oligomeric compound targeted to PPAR-γ, also known to inhibit adipocyte differentiation, may be used as a positive control.

Cells are transfected as described herein. Oligomeric componds are mixed with LIPOFECTIN™ in OPTI-MEM™ to achieve a final concentration of 250 nM of oligomeric compound and 7.5 μg/mL LIPOFECTIN™. Untreated control cells receive LIPOFECTIN™ only. Compounds of the invention and the positive control are tested in triplicate, and the

negative control is tested in up to six replicate wells.

After the cells have reach confluence (approximately three days), they are exposed for an additional three days to differentiation medium (Zen-Bio, Inc., Research Triangle Park, NC) containing a PPAR-γ agonist, IBMX, dexamethasone, and insulin. Cells are then fed adipocyte medium (Zen-Bio, Inc.), which is replaced at 2 or 3 day intervals.

Leptin secretion into the medium in which adipocytes are cultured is measured by protein ELISA. On day nine post-transfection, 96-well plates are coated with a monoclonal antibody to human leptin (R&D Systems, Minneapolis, MN) and left at 4°C overnight. The plates are blocked with bovine serum albumin (BSA), and a dilution of the treated adipoctye medium is incubated in the plate at room temperature for approximately 2 hours. After washing to remove unbound components, a second monoclonal antibody to human leptin (conjugated with biotin) is added. The plate is then incubated with strepavidin-conjugated horse radish peroxidase (HRP) and enzyme levels are determined by incubation with 3, 3', 5, 5'-tetramethlybenzidine, which turns blue when cleaved by HRP. The OD_{450} is read for each well, where the dye absorbance is proportional to the leptin concentration in the cell lysate. Leptin secretion from cells treated with oligomeric compounds is normalized to that from untreated control cells. With respect to leptin secretion, values above or below 100% are considered to indicate that the compound has the ability to stimulate or inhibit leptin secretion, respectively.

The triglyceride accumulation assay measures the synthesis of triglyceride by adipocytes. Triglyceride accumulation is measured using the Infinity™ Triglyceride reagent kit (Sigma-Aldrich, St. Louis, MO). On day nine post-transfection, cells are washed and lysed at room temperature, and the triglyceride assay reagent is added. Triglyceride accumulation is measured based on the amount of glycerol liberated from triglycerides by the enzyme lipoprotein lipase. Liberated glycerol is phosphorylated by glycerol kinase, and hydrogen peroxide is generated during the oxidation of glycerol-1-phosphate to dihydroxyacetone phosphate by glycerol phosphate oxidase. Horseradish peroxidase (HRP) uses H_2O_2 to oxidize 4-aminoantipyrine and 3,5 dichloro-2-hydroxybenzene sulfonate to produce a red-colored dye. Dye absorbance, which is proportional to the concentration of glycerol, is measured at 515 nm using an UV spectrophotometer. Glycerol concentration is calculated from a standard curve for each assay, and data are normalized to total cellular protein as determined by a Bradford assay (Bio-Rad Laboratories, Hercules, CA). Triglyceride accumulation in cells treated with oligomeric compounds is normalized to that in untreated control cells. Values for triglyceride accumulation above or below 100% are considered to indicate that the compound has the ability to stimulate or inhibit triglyceride accumulation, respectively.

Expression of the four hallmark genes, HSL, aP2, Glut4, and PPARγ, is also measured in adipocytes transfected with oligomeric compounds of the invention. Cells are lysed on day nine post-transfection and total RNA is harvested. The amount of total RNA in each sample is determined using a Ribogreen Assay (Invitrogen Life Technologies, Carlsbad, CA). Real-time PCR is performed on the total RNA using primer/probe sets for the adipocyte differentiation hallmark genes Glut4, HSL, aP2, and PPAR-γ. Gene expression in cells treated with oligomeric compounds is normalized to that in untreated control cells. With respect to the four adipocyte differentiation hallmark genes, values above or below 100% are considered to indicate that the compound has the ability to stimulate or inhibit adipocyte differentiation, respectively.

Oligomeric compounds that reduce the expression levels of markers of adipocyte differentiation are candidate therapeutic agents with applications in the treatment, attenuation or prevention of obesity, hyperlipidemia, atherosclerosis, atherogenesis, diabetes, hypertension, or other metabolic diseases as well as having potential applications in the maintenance of the pluripotent phenotype of stem or precursor cells. Oligomeric compounds of the invention resulting in a significant increase in leptin secretion are potentially useful for the treatment of obesity.

Response of liver-derived cells to insulin:

Insulin mediates its effects by suppressing the RNA expression levels of enzymes important for gluconeogenesis and glycogenolysis, and also by controlling the activities of some metabolic enzymes through post-translational mechanisms (Hall and Granner, *J. Basic Clin. Physiol. Pharmacol.,* **1999**, *10*, 119-133; Moller, *Nature,* **2001**, *414*, 821-827; Saltiel and Kahn, *Nature,* **2001**, *414*, 799-806). In liver cells, genes involved in regulating glucose metabolism can be identified by monitoring changes in the expression of selective insulin-responsive genes in a cell culture model. However, primary human hepatocytes are difficult to obtain and work with in culture. Therefore, the insulin signaling assay described herein is performed in the hepatocellular carcinoma cell line HepG2, the most widely used cell culture model for hepatocytes. The insulin responsive genes evaluate in this assay are phosphoenolpyruvate carboxykinase (PEPCK), insulin-like growth factor binding protein 1 (IGFBP-1) and follistatin.

IGFBP-1 is one of a family of six secreted proteins that bind insulin-like growth factor (IGF) with high affinity and thereby modulate IGFs action *in vivo* (Baxter, *Am. J. Physiol. Endocrinol. Metab.,* **2000**, *278*, E967-976; Lee et al., *Proc. Soc. Exp. Biol. Med.,* **1997**, *216*, 319-357). IGFBP-1 is characterized by dynamic variability of levels in circulation due to the regulation of its hepatic secretion (Lee et al., *Proc. Soc. Exp. Biol. Med.,* **1997**, *216*, 319-357). The multi-hormonal regulation of PEPCK and IGFBP-1 are similar. Glucocorticoids and cyclic

AMP (cAMP) stimulate transcription of the IGFBP-1 gene expression whereas insulin acts in a dominant manner to suppress both basal and cAMP or glucocorticoid-stimulated IGFBP-1 gene transcription (O'Brien and Granner, *Physiol. Rev.*, **1996**, *76*, 1109-1161). PEPCK catalyzes the rate-limiting step in gluconeogenesis, and thereby contributes to hepatic glucose output (Hall and Granner, *J. Basic Clin. Physiol. Pharmacol.*, **1999**, *10*, 119-133; Moller, *Nature,* **2001**, *414*, 821-827; Saltiel and Kahn, *Nature,* **2001**, *414*, 799-806). In hepatoma cells, studies have shown that the expression of PEPCK is stimulated by glucocorticoids, glucagon (via cAMP), and retinoic acid. Insulin acts in a dominant manner to suppress these stimulations as well as basal transcription (O'Brien and Granner, *Physiol. Rev.*, **1996**, *76*, 1109-1161). In HepG2 cells, prolonged serum starvation induces the expression of PEPCK and subsequent insulin stimulation significantly reduces the PEPCK mRNA level.

Follistatin is significantly stimulated by insulin in HepG2 cells. Interestingly, follistatin levels have been shown to be higher in women with polycystic ovary syndrome (PCOS) (Norman et al., *Hum. Reprod.*, **2001**, *16*, 668-672). PCOS is a metabolic as well as a reproductive disorder, and an important cause of type 2 diabetes mellitus in women. It is often associated with profound insulin resistance and hyperinsulinemia as well as with a defect in insulin secretion (Dunaif, *Endocr. Rev.*, **1997**, *18*, 774-800; Nestler et al., *Fertil. Steril.*, **2002**, *77*, 209-215). PCOS is the most common cause of female infertility in the U.S. and affects 5%-10% of women of child-bearing age (Dunaif, *Endocr. Rev.*, **1997**, *18*, 774-800; Nestler et al., *Fertil. Steril.*, **2002**, *77*, 209-215).

In some embodiments, HepG2 cells are used to measure the effects of compounds of the invention on hepatic gene expression following insulin stimulation. A 20-nucleotide oligomeric compound with a randomized sequence may be used as a negative control, as it does not target modulators of hepatic gene expression. Insulin at a concentration of 100 nM may be used as a positive control for the stimulation of hepatic gene expression. An oligomeric compound targeted to human forkhead is known to inhibit hepatic gene expression and may be used as a positive control for the inhibition of gene expression in the presence of insulin.

Cells are transfected as described herein. Oligomeric compounds are mixed with LIPOFECTIN™ in OPTI-MEM™ to achieve a final concentration of 100 nM of oligomeric compound and 3 μg/mL LIPOFECTIN™. Untreated control cells receive LIPOFECTIN™ only. Compounds of the invention and the positive controls are tested in triplicate, and the negative control is tested in up to six replicate wells.

Approximately 28 hours after transfection, the cells are subjected to serum starvation for a period of 12 to 16 hours, using serum-free growth medium. Following serum starvation,

cells are treated with 1nM insulin (insulin-treated) or are left untreated (basal conditions) for approximately four hours. At the same time, untreated control cells in both plates are treated with 100 nM insulin to determine the maximal insulin response. Following insulin treatment (forty-eight hours after transfection), total RNA is harvested from all samples, and the amount of total RNA from each sample is determined using a Ribogreen assay (Invitrogen Corporation, Carlsbad, CA). Real-time PCR is performed on the total RNA samples using primer/probe sets for three insulin responsive genes: insulin-like growth factor binding protein-1 (IGFBP-1), cytosolic PEPCK (PEPCK-C), and follistatin. Gene expression levels obtained by real-time PCR are normalized for total RNA content in the samples. Gene expression in cells treated with oligomeric compounds is normalized to that from untreated control cells. Values above or below 100% are considered to indicate an increase or decrease in gene expression, respectively.

Oligomeric compounds that interfere with the expression of genes involved in glucose metabolism are candidate therapeutic agents for the treatment of conditions associated with abnormal glucose metabolism, for example, obesity and diabetes.

Inflammation assays:

In some embodiments, inflammation assays are used. Inflammation assays are designed to identify genes that regulate the activation and effector phases of the adaptive immune response. During the activation phase, T lymphocytes (also known as T-cells) receiving signals from the appropriate antigens undergo clonal expansion, secrete cytokines, and up-regulate their receptors for soluble growth factors, cytokines and co-stimulatory molecules (Cantrell, *Annu. Rev. Immunol.*, **1996**, *14*, 259-274). These changes drive T-cell differentiation and effector function. Response to cytokines by non-immune effector cells controls the production of inflammatory mediators that can do extensive damage to host tissues. The cells of the adaptive immune systems, their products, as well as their interactions with various enzyme cascades involved in inflammation (e.g., the complement, clotting, fibrinolytic and kinin cascades) all represent potential points for intervention in inflammatory disease.

Dendritic cells treated with oligomeric compounds targeting different genes are used to identify regulators of dendritic cell-mediated T-cell co-stimulation. The level of interleukin-2 (IL-2) production by T-cells, a critical consequence of T-cell activation (DeSilva et al., *J. Immunol.*, **1991**, *147*, 3261-3267; Salomon and Bluestone, *Annu. Rev. Immunol.*, **2001**, *19*, 225-252), is used as an endpoint for T-cell activation. T lymphocytes are important immunoregulatory cells that mediate pathological inflammatory responses. Optimal activation of T lymphocytes requires both primary antigen recognition events as well as secondary or co-stimulatory signals from antigen presenting cells (APC). Dendritic cells are the most efficient

APCs known and are principally responsible for antigen presentation to T-cells, expression of high levels of co-stimulatory molecules during infection and disease, and the induction and maintenance of immunological memory (Banchereau and Steinman, *Nature,* **1998**, *392*, 245-252). While a number of co-stimulatory ligand-receptor pairs have been shown to influence T-cell activation, a principal signal is delivered by engagement of CD28 on T-cells by CD80 (B7-1) and CD86 (B7-2) on APCs (Boussiotis et al., *Curr. Opin. Immunol.,* **1994**, *6*, 797-807; Lenschow et al., *Annu. Rev. Immunol.,* **1996**, *14*, 233-258). In contrast, a B7 counter-receptor, CTLA-4, has been shown to negatively regulate T-cell activation, maintain immunological homeostasis and promote immune tolerance (Walunas and Bluestone, *J. Immunol.,* **1998**, *160*, 3855-3860). Inhibition of T-cell co-stimulation by APCs holds promise for novel and more specific strategies of immune suppression. In addition, blocking co-stimulatory signals may lead to the development of long-term immunological anergy (unresponsiveness or tolerance) that would offer utility for promoting transplantation or dampening autoimmunity. T-cell anergy is the direct consequence of failure of T-cells to produce the growth factor interleukin-2 (DeSilva et al., *J. Immunol.,* **1991**, *147*, 3261-3267; Salomon and Bluestone, *Annu. Rev. Immunol.,* **2001**, *19*, 225-252).

Dendritic cell cytokine production as a measure of the activation phase of the immune response:

In some embodiments, the effects of the oligomeric compounds of the invention are examined on the dendritic cell-mediated costimulation of T-cells. A 20-nucleotide oligomeric compound with a randomized sequence may be used as a negative control, as it does not target modulators of dendritic cell-mediated T-cell costimulation. An oligomeric compound targeted to human CD86 is known to inhibit dendritic cell-mediated T-cell stimulation and may be used as a positive control.

Cells are transfected as described herein. Oligomeric compounds are mixed with LIPOFECTIN™ in OPTI-MEM™ to achieve a final concentration of 200 nM of oligomeric compound and 6 μg/mL LIPOFECTIN™. Untreated control cells receive LIPOFECTIN™ only. Compounds of the invention and the positive control are tested in triplicate, and the negative control is tested in up to six replicates. Following incubation with the oligomeric compounds and LIPOFECTIN™, fresh growth medium with cytokines is added and DC culture is continued for an additional 48 hours. DCs are then co-cultured with Jurkat T-cells in RPMI medium (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (Sigma Chemical Company, St. Louis, MO). Culture supernatants are collected 24 hours later and assayed for IL-2 levels (IL-2 DuoSet, R&D Systems, Minneapolis, MN). IL-2 levels in cells treated with oligomeric compounds are normalized to those from untreated control

cells. A value greater than 100% indicates an induction of the inflammatory response, whereas a value less than 100% demonstrates a reduction in the inflammatory response.

Oligomeric compounds that inhibit T-cell co-stimulation are candidate therapeutic compounds with applications in the prevention, treatment or attenuation of conditions associated with hyperstimulation of the immune system, including rheumatoid arthritis, irritable bowel disease, asthma, lupus and multiple sclerosis. Oligomeric compounds that induce T-cell co-stimulation are candidate therapeutic agents for the treatment of immunodeficient conditions. Cytokine signaling as a measure of the effector phase of the inflammatory response:

The cytokine signaling assay further identifies genes that regulate inflammatory responses of non-immune effector cells (initially endothelial cells) to stimulation with cytokines such as interferon-gamma (IFN-γ). Response to IFN-γ is assessed by measuring the expression levels of three genes: intercellular adhesion molecule-1 (ICAM-1), interferon regulatory factor 1 (IRF1) and small inducible cytokine subfamily B (Cys-X-Cys), member 11 (SCYB11). The cytokine signaling assay further identifies genes that regulate inflammatory responses of non-immune effector cells (initially endothelial cells) to stimulation with IL-1β or TNF-α (Heyninck et al., *J Cell Biol,* **1999**, *145*, 1471-1482; Zetoune et al., *Cytokine,* **2001**, *15*, 282-298). Response to IL-1β or TNF-α stimulation is monitored by measuring the expression levels of four genes: A20, intracellular adhesion molecule 1 (ICAM-1), interleukin-9 (IL-8) and macrophage-inflammatory protein 2 (MIP2α). As described below, all of these genes regulate numerous parameters of the inflammatory response.

ICAM-1 is an adhesion molecule expressed at low levels on resting endothelial cells that is markedly up-regulated in response to inflammatory mediators like tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interferon-γ (IFN-γ) (Springer, *Nature,* **1990**, *346*, 425-434). ICAM-1 expression serves to attract circulating leukocytes into the inflammatory site.

IRF-1 binds to upstream cis-regulatory elements of interferon-inducible genes and functions as a transcriptional activator. IRF-1 directly binds to a functional IFN-γ-stimulated response element in the cathepsin S promoter and mediates IFN-γ dependent transcriptional activation (Storm van's Gravesande et al., *J Immunol,* **2002**, *168*, 4488-4494).

SCYB11 is essential for mediating normal leukocyte recruitment and trafficking during inflammation. SCYB11 induces a chemotactic response in IL-2 activated T-cells, monocytes and granulocytes (Mohan et al., *J Immunol,* **2002**, *168*, 6420-6428).

A20 is a zinc-finger protein that limits the transcription of pro-inflammatory genes by blocking TRAF2-stimulated NK-κB signaling. Studies in mice show that TNF-α dramatically increases A20 expression in mice, and that

A20 expression is crucial for their survival (Lee et al., *Science,* **2000**, *289*, 2350-2354).

IL-8 is a member of the chemokine gene superfamily, members of which promote the pro-inflammatory phenotype of macrophages, vascular smooth muscle cells and endothelial cells (Koch et al., *Science,* **1992**, *258*, 1798-1801). IL-8 has been known as one of the major inducible chemokines with the ability to attract neutrophils to the site of inflammation. More recently, IL-8 has been implicated as a major mediator of acute neutrophil-mediated inflammation, and is therefore a potential anti-inflammatory target (Mukaida et al., *Cytokine Growth Factor Rev,* **1998**, *9*, 9-23).

MIP2α, another chemokine known to play a central role in leukocyte extravasation, has more recently been shown to be involved in acute inflammation (Lukacs et al., *Chem Immunol,* **1999**, *72*, 102-120). MIP2α is expressed in response to microbial infection, to injection of lipopolysaccharides (LPS), and to stimulation of cells with pro-inflammatory mediators such as IL-1β and TNF-α (Kopydlowski et al., *J Immunol,* **1999**, *163*, 1537-1544). Endothelial cells are one of several cell types that are sources of MIP2α (Rudner et al., *J Immunol,* **2000**, *164*, 6576-6582).

In some embodiments, the effects of the oligomeric compounds of the invention on the cellular response to cytokines may be examined in HUVECs. A 20-nucleotide oligomeric compound with a randomized sequence may be used as a negative control, as it does not target modulators of cytokine signaling.

Cells are transfected as described herein. Oligomeric compounds are mixed with LIPOFECTIN™ in OPTI-MEM™ to achieve a final concentration of 75 nM of oligomeric compound and 2.25 μg/mL LIPOFECTIN™. Untreated control cells receive LIPOFECTIN™ only. Compounds of the invention are tested in triplicate, and the negative control is tested in up to six replicate wells.

For IFN-γ stimulation, following transfection, fresh growth medium is added and DC culture is continued for an additional 44 hours, after which HUVECS are stimulated with 10 ng/ml of IFN-γ for a period of 4 hours. For stimulation with IL-1β or TNF-α, fresh growth medium is added and DC culture is continued for an additional 46 hours, after which HUVECs are stimulated with 0.1 ng/mL of IL-1β or 1 ng/mL of TNF-α for a period of 2 hours. Total RNA is harvested 48 hours following transfection, and real time PCR is performed using primer/probe sets to detect ICAM-1, IRF-1 and SCYB11 in IFN-γ-stimulated cells, or ICAM-1, A20, IL-8 and MIP2α in IL-1β-stimulated and TNF-α-stimulted cells. Expression levels of each gene are normalized to total RNA. Gene expression levels from cells treated with oligomeric compounds are normalized to those from untreated control cells. A value greater than 100% indicates an

induction of the inflammatory response, whereas a value less than 100% demonstrates a reduction in the inflammatory response.

Oligomeric compounds that inhibit the inflammatory response are candidate therapeutic compounds with applications in the prevention, treatment or attenuation of conditions associated with hyperstimulation of the immune system, including rheumatoid arthritis, irritable bowel disease, asthma, lupus and multiple sclerosis.

In vivo studies

The individual subjects of the *in vivo* studies described herein are warm-blooded vertebrate animals, which includes humans.

Mouse model of tumorigenesis:

Animal models of tumorigenesis are used in some embodiments of the present invention. In this model, tumorigenic cells are injected into immunocompromised mice (i.e. nude mice), and subsequent growth of a tumor is measured.

Serially transplanted MDA-MB-231 (a human breast carcinoma cell line, American Type Culture Collection, Manassus, VA) tumors are established subcutaneously in nude mice. Beginning two weeks later, one or more of the oligomeric compounds of the invention are administered intravenously daily for 14 days at dosages of 15 mg/kg or 30 mg/kg. Control compounds are also administered at these doses, and a saline control is also given. Tumor growth rates are monitored for the two-week period of oligonucleotide administration. Activity of the oligomeric compounds of the invention is measured by a reduction in tumor growth. Activity is measured by reduced tumor volume compared to saline or control compound. Following death or sacrifice of mice, tumor tissue is fixed in 4% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Tumor tissue sections are evaluated for tumor morphology and size.

Human A549 lung tumor cells are also injected into nude mouse to produce tumors. 200 µl of A549 cells (5×10^6 cells) are implanted subcutaneously in the inner thigh of nude mice. Oligomeric compounds of the invention are administered twice weekly for four weeks, beginning one week following tumor cell inoculation. Oligomeric compounds are formulated with cationic lipids (LIPOFECTIN™, Invitrogen Corporation, Carlsbad, CA) and given subcutaneously in the vicinity of the tumor. Oligomeric compound dosage is 5 mg/kg with 60 mg/kg cationic lipid. Tumor size is recorded weekly. Activity of the oligomeric compounds of the invention is measured by reduction in tumor size compared to controls.

Xenograft studies are also performed using the U-87 human glioblastoma cell line (American Type Culture Collection, Manassus, VA). Nude mice are injected subcutaneously

with 2 X 10^7 U-87 cells. Mice are injected intraperitoneally with one or more of the oligomeric compounds of the invention or a control compound at dosages of either 15 mg/kg or 30 mg/kg for 21 consecutive days beginning 7 days after xenografts are implanted. Saline-injected animals serve as a control. Tumor volumes are measured on days 14, 21, 24, 31 and 35. Activity is measured by reduced tumor volume compared to saline or control compound. Following death or sacrifice of mice, tumor tissue is fixed in 4% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Tumor tissue sections are evaluated for tumor morphology and size.

Alternatively, intracerebral U-87 xenografts are generated by implanting U-87 glioblastoma cells into the brains of nude mice. Mice are treated via continuous intraperitoneal administration with one or more of the oligomeric compounds of the invention at 20 mg/kg, control compound at 20 mg/kg or saline beginning on day 7 after xenograft implantation. Activity of the oligomeric compounds of the invention is measured by an increase in survival time compared to controls. Following death or sacrifice, brain tissue is fixed in 4% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Brain tissue sections are evaluated for tumor growth.

Leptin-deficient mice (a model of obesity and diabetes (ob/ob mice)):

Leptin is a hormone produced by fat cells that regulates appetite. Deficiencies in this hormone in both humans and non-human animals leads to obesity. ob/ob mice have a mutation in the leptin gene which results in obesity and hyperglycemia. As such, these mice are a useful model for the investigation of obesity and diabetes and treatments designed to treat these conditions. ob/ob mice have higher circulating levels of insulin and are less hyperglycemic than db/db mice, which harbor a mutation in the leptin receptor. In accordance with the present invention, the oligomeric compounds of the invention are tested in the ob/ob model of obesity and diabetes.

Seven-week old male C57Bl/6J-Lep ob/ob mice (Jackson Laboratory, Bar Harbor, ME) are fed a diet with a fat content of 10-15% and are subcutaneously injected with the oligomeric compounds of the invention or a control compound at a dose of 25 mg/kg two times per week for 4 weeks. Saline-injected animals, leptin wildtype littermates (i.e. lean littermates) and ob/ob mice fed a standard rodent diet serve as controls. After the treatment period, mice are sacrificed and target levels are evaluated in liver, brown adipose tissue (BAT) and white adipose tissue (WAT). RNA isolation and target RNA expression level quantitation are performed as described by other examples herein.

To assess the physiological effects resulting from modulation of target, the ob/ob mice

are evaluated at the end of the treatment period for serum lipids, serum free fatty acids, serum cholesterol (CHOL), liver triglycerides, fat tissue triglycerides and liver enzyme levels. Hepatic steatosis, or clearing of lipids from the liver, is assessed by measuring the liver triglyceride content. Hepatic steatosis is assessed by routine histological analysis of frozen liver tissue sections stained with oil red O stain, which is commonly used to visualize lipid deposits, and counterstained with hematoxylin and eosin, to visualize nuclei and cytoplasm, respectively.

The effects of target modulation on glucose and insulin metabolism are evaluated in the ob/ob mice treated with the oligomeric compounds of the invention. Plasma glucose is measured at the start of the treatment and after 2 weeks and 4 weeks of treatment. Plasma insulin is similarly measured at the beginning of the treatment, and following at 2 weeks and at 4 weeks of treatment. Glucose and insulin tolerance tests are also administered in fed and fasted mice. Mice receive intraperitoneal injections of either glucose or insulin, and the blood glucose and insulin levels are measured before the insulin or glucose challenge and at 15, 20 or 30 minute intervals for up to 3 hours.

To assess the metabolic rate of ob/ob mice treated with the oligomeric compounds of the invention, the respiratory quotient and oxygen consumption of the mice are also measured.

The ob/ob mice that receive treatment are evaluated at the end of the treatment period for the effects of target modulation on the expression of genes that participate in lipid metabolism, cholesterol biosynthesis, fatty acid oxidation, fatty acid storage, gluconeogenesis and glucose metabolism. These genes include, but are not limited to, HMG-CoA reductase, acetyl-CoA carboxylase 1 and acetyl-CoA carboxylase 2, carnitine palmitoyltransferase I and glycogen phosphorylase, glucose-6-phosphatase and phosphoenolpyruvate carboxykinase 1, lipoprotein lipase and hormone sensitive lipase. mRNA levels in liver and white and brown adipose tissue are quantitated by real-time PCR as described in other examples herein, employing primer/probe sets that are generated using published sequences of each gene of interest.

Leptin receptor-deficient mice (a model of obesity and diabetes (db/db mice)):

db/db mice have a mutation in the leptin receptor gene which results in obesity and hyperglycemia. As such, these mice are a useful model for the investigation of obesity and diabetes and treatments designed to treat these conditions. db/db mice, which have lower circulating levels of insulin and are more hyperglycemic than ob/ob mice which harbor a mutation in the leptin gene, are often used as a rodent model of type 2 diabetes. In accordance with the present invention, oligomeric compounds of the present invention are tested in the db/db model of obesity and diabetes.

Seven-week old male C57Bl/6J-Lepr db/db mice (Jackson Laboratory, Bar Harbor, ME) are fed a diet with a fat content of 15-20% and are subcutaneously injected with one or more of the oligomeric compounds of the invention or a control compound at a dose of 25 mg/kg two times per week for 4 weeks. Saline-injected animals, leptin receptor wildtype littermates (i.e. lean littermates) and db/db mice fed a standard rodent diet serve as controls. After the treatment period, mice are sacrificed and target levels are evaluated in liver, BAT and WAT as described *supra*.

To assess the physiological effects resulting from modulation of target, the db/db mice that receive treatment are evaluated at the end of the treatment period for serum lipids, serum free fatty acids, serum cholesterol (CHOL), liver triglycerides, fat tissue triglycerides and liver enzyme levels. Hepatic steatosis is assessed by measuring the liver triglyceride content and oil red O staining, as described *supra*.

The effects of target modulation on glucose and insulin metabolism are also evaluated in the db/db mice treated with the oligomeric compounds of the invention. Plasma glucose is measured at the start of the treatment and after 2 weeks and 4 weeks of treatment. Plasma insulin is similarly measured at the beginning of the treatment, and following 2 weeks and 4 weeks of treatment. Glucose and insulin tolerance tests are also administered in fed and fasted mice. Mice receive intraperitoneal injections of either glucose or insulin, and the blood glucose levels are measured before the insulin or glucose challenge and 15, 30, 60, 90 and 120 minutes following the injection.

To assess the metabolic rate of db/db mice treated with the oligomeric compounds of the invention, the respiratory quotient and oxygen consumption of the mice is also measured.

The db/db mice that receive treatment are evaluated at the end of the treatment period for the effects of target modulation on the expression of genes that participate in lipid metabolism, cholesterol biosynthesis, fatty acid oxidation, fatty acid storage, gluconeogenesis and glucose metabolism, as described *supra*.

Lean mice on a standard rodent diet:

C57Bl/6 mice are maintained on a standard rodent diet and are used as control (lean) animals. In one embodiment of the present invention, the oligomeric compounds of the invention are tested in normal, lean animals.

Seven-week old male C57Bl/6 mice are fed a diet with a fat content of 4% and are subcutaneously injected with one or more of the oligomeric compounds of the invention or control compounds at a dose of 25 mg/kg two times per week for 4 weeks. Saline-injected animals serve as a control. After the treatment period, mice are sacrificed and target levels are

evaluated in liver, BAT and WAT as described *supra*.

To assess the physiological effects resulting from modulation of the target, the lean mice that receive treatment are evaluated at the end of the treatment period for serum lipids, serum free fatty acids, serum cholesterol (CHOL), liver triglycerides, fat tissue triglycerides and liver enzyme levels. Hepatic steatosis is assessed by measuring the liver triglyceride content and oil red O staining, as described *supra*.

The effects of target modulation on glucose and insulin metabolism are also evaluated in the lean mice treated with the oligomeric compounds of the invention. Plasma glucose is measured at the start of the treatment and after 2 weeks and 4 weeks of treatment. Plasma insulin is similarly measured at the beginning of the treatment, and following 2 weeks and 4 weeks of treatment. Glucose and insulin tolerance tests are also administered in fed and fasted mice. Mice receive intraperitoneal injections of either glucose or insulin, and the blood glucose levels are measured before the insulin or glucose challenge and 15, 30, 60, 90 and 120 minutes following the injection.

To assess the metabolic rate of lean mice treated with the oligomeric compounds of the invention, the respiratory quotient and oxygen consumption of the mice is also measured.

The lean mice that received treatment are evaluated at the end of the treatment period for the effects of target modulation on the expression of genes that participate in lipid metabolism, cholesterol biosynthesis, fatty acid oxidation, fatty acid storage, gluconeogenesis and glucose metabolism, as described *supra*.

Levin Model of diet-induced obesity in rats:

The Levin Model is a polygenic model of rats selectively bred to develop diet-induced obesity (DIO) associated with impaired glucose tolerance, dyslipidemia and insulin resistance when fed a high-fat diet (Levin, et al., *Am. J. Physiol*, **1997**, 273, R725-30). The advantage of this model is that it displays traits more similar to human obesity and glucose intolerance than in animals that are obese/ hyperinsulinemic due to genetic defects e.g. defects in leptin signaling. This model is useful in investigating the oligomeric compounds of the present invention for their ability to affect obesity and related complications, such as impaired glucose tolerance, dyslipidemia and insulin resistance. In accordance with the present invention, the oligomeric compounds of the invention are tested in the Levin model of diet-induced obesity.

Eight-week old male Levin rats (Charles River Laboratories, Wilmington, MA), weighing ~500 g, are fed a diet with a fat content of 60% for eight weeks, after which they are subcutaneously injected with one or more of the oligomeric compounds of the invention at a dose of 25 mg/kg X 2 per week for 8 weeks. Control groups consist of animals injected with

saline or a control compound and lean littermates fed a standard rodent diet. The control compound is injected at the same dose as the target-specific compound.

Throughout the treatment period, the rats are evaluated for food consumption, weight gain, as well as serum levels of glucose, insulin, cholesterol, free fatty acids, triglycerides and liver enzymes.

The effects of target modulation on glucose and insulin metabolism are also evaluated in the Levin rats treated with the oligomeric compounds of the invention. Plasma glucose and insulin are monitored throughout the treatment by analyzing blood samples. Glucose and tolerance are assessed in fed or fasted rats. After blood is collected for baseline glucose and insulin levels, a glucose challenge is administered, after which blood glucose and insulin levels are measured at 15, 20 or 30 minute intervals for up to 3 hours. Insulin tolerance is similarly analyzed, beginning with blood collection for baseline glucose and insulin levels, followed by an insulin challenge, after which blood glucose levels are measured at 15, 20 or 30 minute intervals for up to 3 hours. Plasma insulin and glucose are also measured at study termination.

At the end of the treatment period, the rats are sacrificed. Organs are removed and weighed, including liver, white adipose tissue, brown adipose tissue and spleen. Target RNA expression levels are measured in all tissues that are isolated, using quantitative real-time PCR. Target protein levels are also evaluated by immunoblot analysis using antibodies that specifically recognize the target protein.

Also evaluated at the end of the treatment period are serum lipids, serum free fatty acids, serum cholesterol (CHOL), liver triglycerides, fat tissue triglycerides and liver enzyme levels. Hepatic steatosis is assessed by measuring the liver triglyceride content and oil red O staining, as described *supra*.

The Levin rats that receive treatment are evaluated at the end of the treatment period for the effects of target modulation on the expression of genes that participate in lipid metabolism, cholesterol biosynthesis, fatty acid oxidation, fatty acid storage, gluconeogenesis and glucose metabolism, as described *supra*.

C57BL/6 on a high-fat diet (a model of diet-induced obesity (DIO)):

The C57BL/6 mouse strain is reported to be susceptible to hyperlipidemia-induced atherosclerotic plaque formation. Consequently, when these mice are fed a high-fat diet, they develop diet-induced obesity. Accordingly these mice are a useful model for the investigation of obesity and treatments designed to treat these conditions. In one embodiment of the present invention, the oligomeric compounds of the invention are tested in a model of diet-induced obesity.

Male C57BL/6 mice (7-weeks old) receive a 60% fat diet for 8 weeks, after which mice are subcutaneously injected with one or more of the oligomeric compounds of the invention at a dose of 25 mg/kg two times per week for 4 weeks. Saline-injected or control compound-injected animals serve as a control. After the treatment period, mice are sacrificed and target levels are evaluated in liver, BAT and WAT as described *supra*.

To assess the physiological effects resulting from modulation of target, the diet-induced obese mice that receive treatment are evaluated at the end of the treatment period for serum lipids, serum free fatty acids, serum cholesterol (CHOL), liver triglycerides, fat tissue triglycerides and liver enzyme levels. Hepatic steatosis is assessed by measuring the liver triglyceride content and oil red O staining, as described *supra*.

The effects of target modulation on glucose and insulin metabolism are also evaluated in the diet-induced obese mice treated with the oligomeric compounds of the invention. Plasma glucose is measured at the start of treatment and after 2 weeks and 4 weeks of treatment. Plasma insulin is similarly measured at the beginning of the treatment, and following 2 weeks and 4 weeks of treatment. Glucose and insulin tolerance tests are also administered in fed and fasted mice. Mice receive intraperitoneal injections of either glucose or insulin, and the blood glucose and insulin levels are measured before the insulin or glucose challenge and at 15, 20 or 30 minute intervals for up to 3 hours.

To assess the metabolic rate of diet-induced obese mice treated with the oligomeric compounds of the invention, the respiratory quotient and oxygen consumption of the mice is also measured.

The diet-induced obese mice that receive treatment are evaluated at the end of the treatment period for the effects of target modulation on the expression of genes that participate in lipid metabolism, cholesterol biosynthesis, fatty acid oxidation, fatty acid storage, gluconeogenesis and glucose metabolism, as described *supra*.

P-407 mouse model of hyperlipidemia:

Poloxamer 407 (P-407), an inert block copolymer comprising a hydrophobic core flanked by hydrophilic polyoxyethelene units has been shown to induce hyperlipidemia in rodents. In the mouse, one injection, intraperitoneally, of P-407 (0.5g/kg) produced hypercholesterolemia that peaked at 24 hours and returned to control levels by 96 hours following treatment (Palmer, et al., *Atherosclerosis,* **1998**, *136*, 115-123). Consequently, these mice are a useful model for the investigation of compounds that modulate hyperlipidemia. In accordance with the present invention, the oligomeric compounds of the invention are tested in the P-407 model of hyperlipidemia.

Seven-week old male C57Bl/6 mice are divided into two groups; (1) control and (2) P-407 injected animals (.5 g/kg every 3 days, following an overnight fast). Animals in each group receive either a saline injection or injection with one or more of the oligomeric compounds of the invention or control compounds at 25 mg/kg three times per week or 50 mg/kg two times per week. All injections are administered intraperitoneally.

After the treatment period, mice are sacrificed and target levels are evaluated in liver, BAT and WAT as described *supra*.

To assess the physiological effects resulting from modulation of target, the P-407 injected animals that receive treatment are evaluated at the end of the treatment period for serum lipids, serum free fatty acids, serum cholesterol (CHOL), liver triglycerides, fat tissue triglycerides and liver enzyme levels. Hepatic steatosis is assessed by measuring the liver triglyceride content and oil red O staining, as described *supra*.

The effects of target modulation on glucose and insulin metabolism are evaluated in the P-407 injected animals treated with the oligomeric compounds of the invention. Plasma glucose is measured at the start of the treatment and after 2 weeks and 4 weeks of treatment. Plasma insulin is similarly measured at the beginning of the treatment, and following 2 weeks and 4 weeks of treatment. Glucose and insulin tolerance tests are also administered in fed and fasted mice. Mice receive intraperitoneal injections of either glucose or insulin, and the blood glucose and insulin levels are measured before the insulin or glucose challenge and at 15, 20 or 30 minute intervals for up to 3 hours.

To assess the metabolic rate of P-407 injected animals treated with the oligomeric compounds of the invention, the respiratory quotient and oxygen consumption of the mice is measured.

The P-407 injected animals that receive treatment are evaluated at the end of the treatment period for the effects of target modulation on the expression of genes that participate in lipid metabolism, cholesterol biosynthesis, fatty acid oxidation, fatty acid storage, gluconeogenesis and glucose metabolism, as described *supra*.

ApoE knockout mice (a model of dyslipidemia and obesity):

B6.129P-ApoE^{tm1Unc} knockout mice (herein referred to as ApoE knockout mice) obtained from The Jackson Laboratory (Bar Harbor, ME), are homozygous for the *Apoe^{tm1Unc}* mutation and show a marked increase in total plasma cholesterol levels that are unaffected by age or sex. These animals present with fatty streaks in the proximal aorta at 3 months of age. These lesions increase with age and progress to lesions with less lipid but more elongated cells, typical of a more advanced stage of pre-atherosclerotic lesion.

The mutation in these mice resides in the apolipoprotein E (ApoE) gene. The primary role of the ApoE protein is to transport cholesterol and triglycerides throughout the body. It stabilizes lipoprotein structure, binds to the low density lipoprotein receptor (LDLR) and related proteins, and is present in a subclass of HDLs, providing them the ability to bind to LDLR. ApoE is expressed most abundantly in the liver and brain. In one embodiment of the present invention, female B6.129P-Apoetm1Unc knockout mice (ApoE knockout mice) are used in the following studies to evaluate the oligomeric compounds of the invention as potential lipid lowering compounds.

Female ApoE knockout mice range in age from 5 to 7 weeks and are placed on a normal diet for 2 weeks before study initiation. ApoE knockout mice are then fed *ad libitum* a 60% fat diet, with 0.15% added cholesterol to induce dyslipidemia and obesity. Control animals include ApoE knockout mice and ApoE wildtype mice (i.e. lean littermates) maintained on a high-fat diet with no added cholesterol. After overnight fasting, mice from each group are dosed intraperitoneally every three days with saline, 50 mg/kg of a control compound or 5, 25 or 50 mg/kg of one or more of the oligomeric compounds of the invention.

After the treatment period, mice are sacrificed and target levels are evaluated in liver, BAT and WAT as described *supra*.

To assess the physiological effects resulting from modulation of target, the ApoE knockout mice that receive treatment are evaluated at the end of the treatment period for serum lipids, serum free fatty acids, serum cholesterol (CHOL), liver triglycerides, fat tissue triglycerides and liver enzyme levels. Hepatic steatosis is assessed by measuring the liver triglyceride content and oil red O staining, as described *supra*.

The effects of target modulation on glucose and insulin metabolism are also evaluated in the ApoE knockout mice treated with the oligomeric compounds of the invention. Plasma glucose is measured at the start of the treatment and after 2 weeks and 4 weeks of treatment. Plasma insulin is similarly measured at the beginning of the treatment, and following 2 weeks and 4 weeks of treatment. Glucose and insulin tolerance tests are also administered in fed and fasted mice. Mice receive intraperitoneal injections of either glucose or insulin, and the blood glucose and insulin levels are measured before the insulin or glucose challenge and at 15, 20 or 30 minute intervals for up to 3 hours.

To assess the metabolic rate of ApoE knockout mice treated with the oligomeric compounds of the invention, the respiratory quotient and oxygen consumption of the mice are measured.

The ApoE knockout mice that receive treatment are evaluated at the end of the

treatment period for the effects of target modulation on the expression of genes that participate in lipid metabolism, cholesterol biosynthesis, fatty acid oxidation, fatty acid storage, gluconeogenesis and glucose metabolism, as described *supra*.

In order that the invention disclosed herein may be more efficiently understood, examples are provided below. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the invention in any manner. Throughout these examples, molecular cloning reactions, and other standard recombinant DNA techniques, were carried out according to methods described in Maniatis et al., Molecular Cloning - A Laboratory Manual, 2nd ed., Cold Spring Harbor Press (1989), using commercially available reagents, except where otherwise noted.

EXAMPLES

Example 1: Oligomeric compounds targeting small non-coding RNAs

In accordance with the present invention, a series of oligomeric compounds are designed to target different regions of small non-coding target RNAs. The oligomeric compounds can be investigated for their effect on small non-coding RNA levels by quantitative real-time PCR. The target regions to which these sequences are complementary are herein referred to as "suitable target regions".

Example 2: Oligomeric compounds that mimic or replace small non-coding RNAs

In accordance with the present invention, a series of oligomeric compounds are designed to mimic the structure and/or function of small non-coding RNAs. These mimics may include isolated single-, double-, or multiple-stranded compounds, any of which may include regions of intrastrand nucleobase complementarity, said regions capable of folding and forming a molecule with fully or partially double-stranded or multiple-stranded character based on regions of precise or imperfect complementarity. The oligomeric compound mimics can then be investigated for their effects on a cell, tissue or organism system lacking endogenous small non-coding RNAs or systems with aberrant expression of small non-coding RNAs using the screening methods disclosed herein or those commonly used in the art. Changes in levels, expression or function of the small non-coding RNA or its downstream target nucleic acid levels can be analyzed by quantitative real-time PCR as described, *supra*.

Example 3: Pri-miRNAs targeted by compounds of the present invention

In accordance with the present invention, oligomeric compounds were designed to target one or more microRNA (miRNA) genes or gene products. Certain pri-miRNAs have been reported by Lim et al. *Science*, **2003**, 299; 1540; *in Brevia* (detailed in the supplemental online materials; www.sciencemag.org/cgi/content/full/299/5612/1540/DC1) and these were used as starting targets.

A list of pri-miRNAs targeted is shown in Table 1. The gene name for each of the 188 targets (assigned by Lim et al.) is given in the table. For those pri-miRNAs that did not produce an identifiable miRNA detectable by PCR in the Lim publication, the position and sequence of the miRNAs were identified herein and are referred to as novel or hypothetical miRNAs. Also shown are the sequence of the pri-miRNA and the Genbank Accession number of the source sequence from which the pri-miRNA was extracted. The sequence is written in the 5' to 3' direction and is represented in the DNA form. It is understood that a person having ordinary skill in the art would be able to convert the sequence of the targets to their RNA form by simply replacing the thymidine (T) with uracil (U) in the sequence.

Table 1

pri-miRNAs

pri-miRNA	pri-miRNA sequence	Genbank Accession # of source sequence	SEQ ID NO
mir-140	CTGTGTGTGTCTCTCTCTGTGTCCTGCCAG TGGTTTTACCCTATGGTAGGTTACGTCATG CTGTTCTACCACAGGGTAGAACCACGGACA GGATACCGGGGCACCCTCTG	NT_037896.1	4
mir-30a	TATATTGCTGTTGACAGTGAGCGACTGTAA ACATCCTCGACTGGAAGCTGTGAAGCCACA GATGGGCTTTCAGTCGGATGTTTGCAGCTG CCTACTGCCTCGGACTTCAA	NT_007299.11	5
mir-34	GGCCAGCTGTGAGTGTTTCTTTGGCAGTGT CTTAGCTGGTTGTTGTGAGCAATAGTAAGG AAGCAATCAGCAAGTATACTGCCCTAGAAG TGCTGCACGTTGTGGGGCCC	NT_028054.10	6
mir-29b-1	TCATTGAGATCCTCTTCTTCTGGAAGCTGG TTTCACATGGTGGCTTAGATTTTTCCATCT TTGTATCTAGCACCATTTGAAATCAGTGTT TTAGGAGTAAGAATTGCAGC	NT_021877.13	7
mir-29b-2	GATCATAAAGCTTCTTCAGGAAGCTGGTTT CATATGGTGGTTTAGATTTAAATAGTGATT GTCTAGCACCATTTGAAATCAGTGTTCTTG GGGGAGACCAGCTGCGCTGC	NT_007933.10	8
mir-16-3	ATGAACTGACATACTTGTTCCACTCTAGCA GCACGTAAATATTGGCGTAGTGAAATATAT ATTAAACACCAATATTACTGTGCTGCTTTA GTGTGACAGGGATACAGCAA	NT_005612.11	9
mir-203	GTGTTGGGGACTCGCGCGCTGGGTCCAGTG GTTCTTAACAGTTCAACAGTTCTGTAGCGC	NT_026437.9	10

	AATTGTGAAATGTTTAGGACCACTAGACCC GGCGGGCGCGGCGACAGCGA		
mir-7-1	TTGGATGTTGGCCTAGTTCTGTGTGGAAGA CTAGTGATTTTGTTGTTTTTAGATAACTAA ATCGACAACAAATCACAGTCTGCCATATGG CACAGGCCATGCCTCTACAG	NT_023935.13	11
mir-10b	CCAGAGGTTGTAACGTTGTCTATATATACC CTGTAGAACCGAATTTGTGTGGTATCCGTA TAGTCACAGATTCGATTCTAGGGGAATATA TGGTCGATGCAAAAACTTCA	NT_037537.1	12
mir-128a	TGCAATAATTGGCCTTGTTCCTGAGCTGTT GGATTCGGGGCCGTAGCACTGTCTGAGAGG TTTACATTTCTCACAGTGAACCGGTCTCTT TTTCAGCTGCTTCCTGGCTT	NT_034487.2	13
mir-153-1	TctctctctcccctcACAGCTGCCAGTGTCA TTTTTGTGATCTGCAGCTAGTATTCTCACT CCAGTTGCATAGTCACAAAAGTGATCATTG GCAGGTGTGGCTGCTGCATG	NT_005403.11	14
mir-153-2	TGCCAGCTAATTAGCGGTGGCCAGTGTCAT TTTTGTGATGTTGCAGCTAGTAATATGAGC CCAGTTGCATAGTCACAAAAGTGATCATTG GAAACTGTGACTGTACTGCA	NT_007741.10	15
hypothetical miRNA-013	CTGGATGCCTTTTCTGCAGGCCTCTGTGTG ATATGTTTGATATATTAGGTTGTTATTTAA TCCAACTATATATCAAACATATTCCTACAG TGTCTTGCCCTGTCTCCGGG	NT_010194.13	16
mir-27b	TGACCTCTCTAACAAGGTGCAGAGCTTAGC TGATTGGTGAACAGTGATTGGTTTCCGCTT TGTTCACAGTGGCTAAGTTCTGCACCTGAA GAGAAGGTGAGATGGGGACA	NT_008476.13	17
mir-96	CAGTGCCATCTGCTTGGCCGATTTTGGCAC TAGCACATTTTTGCTTGTGTCTCTCCGCTC TGAGCAATCATGTGCAGTGCCAATATGGGA AAAGCAGGACCCGCAGCTGC	NT_007933.10	18
mir-17as/mir-91	AGTCAGAATAATGTCAAAGTGCTTACAGTG CAGGTAGTGATATGTGCATCTACTGCAGTG AAGGCACTTGTAGCATTATGGTGACAGCTG CCTCGGGAAGCCAAGTTGGG	NT_009952.11	19
mir-123/mir-126as	GCCACGCCTCCGCTGGCGACGGGACATTAT TACTTTTGGTACGCGCTGTGACACTTCAAA CTCGTACCGTGAGTAATAATGCGCCGTCCA CGGCACCGCATCGAAAACGC	NT_024000.13	20
mir-132	CgcgccccgcccccgcGTCTCCAGGGCAAC CGTGGCTTTCGATTGTTACTGTGGGAACTG GAGGTAACAGTCTACAGCCATGGTCGCCCC GCAGCACGCCCACGCGCCGC	NT_010692.9	21
mir-108-1	GCTGCCCGATGCACACTGCAAGAACAATAA GGATTTTTAGGGGCATTATGACTGAGTCAG AAAACACAGCTGCCCCTGAAAGTCCCTCAT TTTTCTTGCTGTCCTTGAAC	NT_010799.11	22
mir-23b	GCGCTGCTCTCAGGTGCTCTGGCTGCTTGG GTTCCTGGCATGCTGATTTGTGACTTAAGA TTAAAATCACATTGCCAGGGATTACCACGC AACCACGACCTTGGCTGCTC	NT_008476.13	23
let-7i	ACACCATGGCCCTGGCTGAGGTAGTAGTTT GTGCTGTTGGTCGGGTTGTGACATTGCCCG CTGTGGAGATAACTGCGCAAGCTACTGCCT TGCTAGTGCTGGTGATGCTC	NT_009711.13	24
mir-212	CGGGGCACCCCGCCCGGACAGCGCGCCGGC ACCTTGGCTCTAGACTGCTTACTGCCCGGG CCGCCCTCAGTAACAGTCTCCAGTCACGGC	NT_010692.9	25

	CACCGACGcctggccccgcc		
hypothetical miRNA-023	AGATTTAATTAGCTCAGAGAAGAAATGTTG CTTGGGCAAGAGGACTTTTTAATTATCAGC TTGGATAAATTTGAAAATGTTGATGCCTAG GGGTTGAGTTAATTAAAACC	NT_004658.12	26
mir-131-2	GCCTGTGTGGGAAGCGAGTTGTTATCTTTG GTTATCTAGCTGTATGAGTGTATTGGTCTT CATAAAGCTAGATAACCGAAAGTAAAAACT CCTTCAAGATCGCCGGGGAG	NT_029973.6	27
let-7b	GGCCGGGCCTGGCGGGGTGAGGTAGTAGGT TGTGTGGTTTCAGGGCAGTGATGTTGCCCC TCGGAAGATAACTATACAACCTACTGCCTT CCCTGAGGAGCCCAGTGACA	NT_011523.8	28
mir-1d	CTGCATGCAGACTGCCTGCTTGGGAAACAT ACTTCTTTATATGCCCATATGGACCTGCTA AGCTATGGAATGTAAAGAAGTATGTATCTC AGGCCGGGACCTCTCTCGCC	NT_035608.1	29
mir-122a	TGGCTACAGAGTTTCCTTAGCAGAGCTGTG GAGTGTGACAATGGTGTTTGTGTCTAAACT ATCAAACGCCATTATCACACTAAATAGCTA CTGCTAGGCAATCCTTCCCT	NT_033907.3	30
mir-22	GCCCTCACCTGGCTGAGCCGCAGTAGTTCT TCAGTGGCAAGCTTTATGTCCTGACCCAGC TAAAGCTGCCAGTTGAAGAACTGTTGCCCT CTGCCCCTGGCTTCGAGGAG	NT_010692.9	31
mir-92-1	AAGGGAAACTCAAACCCCTTTCTACACAGG TTGGGATCGGTTGCAATGCTGTGTTTCTGT ATGGTATTGCACTTGTCCCGGCCTGTTGAG TTTGGTGGGGATTGTGACCA	NT_009952.11	32
hypothetical miRNA-030	CTACTGCTGTTGGTGGCAGCTTGGTGGTCG TATGTGTGACGCCATTTACTTGAACCTTTA GGAGTGACATCACATATACGGCAGCTAAAC TGCTACATGGGACAACAATT	NT_007933.10	33
mir-142	CGACGGACAGACAGACAGTGCAGTCACCCA TAAAGTAGAAAGCACTACTAACAGCACTGG AGGGTGTAGTGTTTCCTACTTTATGGATGA GTGTACTGTGGGCTTCGGAG	NT_010783.11	34
mir-183	CCGCAGAGTGTGACTCCTGTTCTGTGTATG GCACTGGTAGAATTCACTGTGAACAGTCTC AGTCAGTGAATTACCGAAGGGCCATAAACA GAGCAGAGACAGATCCACGA	NT_007933.10	35
hypothetical miRNA-033	TGGTGTGGCAACCCCTAAAGGCTCAGCATT AAGGTGGGTGGAATAATATAACAATATCCG TGTTGTTATAGTATTCCACCTACCCTGATG CATTTTGTTGTCATTTTCTT	NT_011588.11	36
mir-214	GGCCTGGCTGGACAGAGTTGTCATGTGTCT GCCTGTCTACACTTGCTGTGCAGAACATCC GCTCACCTGTACAGCAGGCACAGACAGGCA GTCACATGACAACCCAGCCT	NT_029874.7	37
mir-143	AGCAGCGCAGCGCCCTGTCTCCCAGCCTGA GGTGCAGTGCTGCATCTCTGGTCAGTTGGG AGTCTGAGATGAAGCACTGTAGCTCAGGAA GAGAGAAGTTGTTCTGCAGC	NT_006859.11	38
mir-192-1	GCCGAGACCGAGTGCACAGGGCTCTGACCT ATGAATTGACAGCCAGTGCTCTCGTCTCCC CTCTGGCTGCCAATTCCATAGGTCACAGGT ATGTTCGCCTCAATGCCAGC	NT_033241.3	39
mir-192-2	GCCGAGACCGAGTGCACAGGGCTCTGACCT ATGAATTGACAGCCAGTGCTCTCGTCTCCC CTCTGGCTGCCAATTCCATAGGTCACAGGT ATGTTCGCCTCAATGCCAGC	NT_033241.3	39

mir-192-3	GCCGAGACCGAGTGCACAGGGCTCTGACCT ATGAATTGACAGCCAGTGCTCTCGTCTCCC CTCTGGCTGCCAATTCCATAGGTCACAGGT ATGTTCGCCTCAATGCCAGC	NT_033241.3	39
hypothetical miRNA-039	CCCCTGTGCCTTGGGCGGGCGGCTGTTAAG ACTTGCAGTGATGTTTAACTCCTCTCCACG TGAACATCACAGCAAGTCTGTGCTGCTTCC CGTCCCTACGCTGCCTGGGC	NT_028392.4	42
hypothetical miRNA-040	GCCAGCAAATAATGGCTGTTGTATTAGCTG CTTTTGATGATAGTATGAAAGAAGTATTAG CACTTGTCAACAAAACTGCTTACAACATAA CATTAGCATGCATGGGCTGC	NT_023148.9	43
hypothetical miRNA-041	CATACACGGCTGTTACACAGGTTTTCCCAT GATAAGGCAATAGGTTAATGAAATGCTCAT TTCATTTTACCAGTTGTTTTCTCTGTGAAG TTCCGATAAGTAGCAAACCA	NT_023089.11	44
let-7a-3	CGACTGCCCTTTGGGGTGAGGTAGTAGGTT GTATAGTTTGGGGCTCTGCCCTGCTATGGG ATAACTATACAATCTACTGTCTTTCCTGAA GTGGCTGTAATATCTGCGGT	NT_011523.8	45
hypothetical miRNA-043	CCCCTTATAGGCCCATTTTGACAGGAAATC TTTGAGAGGCAGCAGCAATGAAGTGCCCAG AGATTTCATCTGTCTTCTTTTGCTTTAGGA AATGCTGAGCGCAAGGCTCC	NT_004902.12	46
hypothetical miRNA-044	GCCTGAAATGAAATTACCATATTTTTAATC TTAATTTTCCACTCTGTTTATCTGACAGTG TGGATGTGCAATCCAAACAGATAATGAGAG AGTGGGATATTGACACCGCT	NT_009952.11	47
mir-181a	AGAAGGGCTATCAGGCCAGCCTTCAGAGGA CTCCAAGGAACATTCAACGCTGTCGGTGAG TTTGGGATTTGAAAAAACCACTGACCGTTG ACTGTACCTTGGGGTCCTTA	NT_017568.11	48
let-7a-1	GTTCTCTTCACTGTGGGATGAGGTAGTAGG TTGTATAGTTTTAGGGTCACACCCACCACT GGGAGATAACTATACAATCTACTGTCTTTC CTAACGTGATAGAAAAGTCT	NT_008476.13	49
mir-205	AAAGATCCTCAGACAATCCATGTGCTTCTC TTGTCCTTCATTCCACCGGAGTCTGTCTCA TACCCAACCAGATTTCAGTGGAGTGAAGTT CAGGAGGCATGGAGCTGACA	NT_021877.13	50
mir-103-1	GAAGTTTTCTTACTGCCCTCGGCTTCTTTA CAGTGCTGCCTTGTTGCATATGGATCAAGC AGCATTGTACAGGGCTATGAAGGCATTGAG ACCTGTTCTTCATGATATTT	NT_037665.1	51
mir-26a	GGCCCTGGCGAAGGCCGTGGCCTCGTTCAA GTAATCCAGGATAGGCTGTGCAGGTCCCAA TGGCCTATCTTGGTTACTTGCACGGGGACG CGGGCCTGGACGCCGGCATC	NT_005580.13	52
mir-33a	GGCCGCATACCTCCTGGCGGGCAGCTGTGG TGCATTGTAGTTGCATTGCATGTTCTGGTG GTACCCATGCAATGTTTCCACAGTGCATCA CAGAGGCCTGCCTGGCCCTC	NT_011520.8	53
mir-196-2	TGCTCGCTCAGCTGATCTGTGGCTTAGGTA GTTTCATGTTGTTGGGATTGAGTTTTGAAC TCGGCAACAAGAAACTGCCTGAGTTACATC AGTCGGTTTTCGTCGAGGGC	NT_009458.12	54
mir-107	TGCAATATTCGATATTCTCTCTGCTTTCAG CTTCTTTACAGTGTTGCCTTGTGGCATGGA GTTCAAGCAGCATTGTACAGGGCTATCAAA GCACAGAGAGCTTGCTACAG	NT_033890.3	55
mir-106	AAATTTTGCTACAGGAATAGGCCTTGGCCA	NT_011786.11	56

	TGTAAAAGTGCTTACAGTGCAGGTAGCTTT TTGAGATCTACTGCAATGTAAGCACTTCTT ACATTACCATGGTGATTTAG		
let-7f-1	ATTGCTCTATCAGAGTGAGGTAGTAGATTG TATAGTTGTGGGGTAGTGATTTTACCCTGT TCAGGAGATAACTATACAATCTATTGCCTT CCCTGAGGAGTAGACTTGCT	NT_008476.13	57
hypothetical miRNA-055	TTGAGCATGATGAATGATTGGAGTCAGAGA AGCGGCGTGATAAATGGCAGCACCTTGGCT CCATTGCATGCCCTATTGATTCTCCTTCTT TATTACTCCTACAACCCAGC	NT_006713.11	58
mir-29c	ACCACTGGCCCATCTCTTACACAGGCTGAC CGATTTCTCCTGGTGTTCAGAGTCTGTTTT TGTCTAGCACCATTTGAAATCGGTTATGAT GTAGGGGGAAAAGCAGCAGC	NT_021877.13	59
mir-130a	CCGGCATGCCTCTGCTGCTGGCCAGAGCTC TTTTCACATTGTGCTACTGTCTGCACCTGT CACTAGCAGTGCAATGTTAAAAGGGCATTG GCCGTGTAGTGCTACCCAGC	NT_033903.3	60
hypothetical miRNA-058	TATCATCTTGTCAGATGCTTAATGTTCTTC CTCCTGTCACTTTGGATAGGCCCAATTTGT AGAATACTGCAGAGGTAAAAGAAGACAATT AACAGTGACAGGATGGTAAT	NT_037537.1	61
mir-218-1	GTGATAATGTAGCGAGATTTTCTGTTGTGC TTGATCTAACCATGTGGTTGCGAGGTATGA GTAAAACATGGTTCCGTCAAGCACCATGGA ACGTCACGCAGCTTTCTACA	NT_006316.13	62
mir-124a-2	ATCAAGATTAGAGGCTCTGCTCTCCGTGTT CACAGCGGACCTTGATTTAATGTCATACAA TTAAGGCACGCGGTGAATGCCAAGAGCGGA GCCTACGGCTGCACTTGAAG	NT_008183.13	63
mir-21	CTCCATGGCTGTACCACCTTGTCGGGTAGC TTATCAGACTGATGTTGACTGTTGAATCTC ATGGCAACACCAGTCGATGGGCTGTCTGAC ATTTTGGTATCTTTCATCTG	NT_035426.2	64
mir-16-1	AGCTCTTATGATAGCAATGTCAGCAGTGCC TTAGCAGCACGTAAATATTGGCGTTAAGAT TCTAAAATTATCTCCAGTATTAACTGTGCT GCTGAAGTAAGGTTGACCAT	NT_033922.3	65
mir-144	TCCTGTGCCCCCAGTGGGGCCCTGGCTGGG ATATCATCATATACTGTAAGTTTGCGATGA GACACTACAGTATAGATGATGTACTAGTCC GGGCACCCCCAGCTCTGGAG	NT_010799.11	66
mir-221	TGAACATCCAGGTCTGGGGCATGAACCTGG CATACAATGTAGATTTCTGTGTTCGTTAGG CAACAGCTACATTGTCTGCTGGGTTTCAGG CTACCTGGAAACATGTTCTC	NT_011568.10	67
mir-222	GCTGCTGGAAGGTGTAGGTACCCTCAATGG CTCAGTAGCCAGTGTAGATCCTGTCTTTCG TAATCAGCAGCTACATCTGGCTACTGGGTC TCTGATGGCATCTTCTAGCT	NT_011568.10	68
mir-30d	TCTTGTTCAGAAAGTCTGTTGTTGTAAACA TCCCCGACTGGAAGCTGTAAGACACAGCTA AGCTTTCAGTCAGATGTTTGCTGCTACCGG CTATTCACAGACATCCTCTT	NT_028251.8	69
mir-19b-2	CAAAGACATTGCTACTTACAATTAGTTTTG CAGGTTTGCATTTCAGCGTATATATGTATA TGTGGCTGTGCAAATCCATGCAAAACTGAT TGTGATAATGTGTGCTTCCT	NT_011786.11	70
mir-128b	GCCCGGCAGCCACTGTGCAGTGGGAAGGGG GGCCGATACACTGTACGAGAGTGAGTAGCA	NT_005580.13	71

	GGTCTCACAGTGAACCGGTCTCTTTCCCTA CTGTGTCACACTCCTAATGG		
hypothetical miRNA-069	GGGCCCTGAACTCAGGGGCTTCGCCACTGA TTGTCCAAACGCAATTCTTGTACGAGTCTG CGGCCAACCGAGAATTGTGGCTGGACATCT GTGGCTGAGCTCCGGGCGCA	NT_017568.11	72
hypothetical miRNA-070	TTCGATGCTTGAAGATGTCAGACTGTAGAA TCTCTACGGGTAAGTGTGTGATTTCCTCAG TGACATCACATTTGCCTGCAGAGATTTTCC AGTCTGCCACTTTGAAGTTG	NT_005375.11	73
hypothetical miRNA-071	GCTTGCTGTAGGCTGTATGCTGTTAATGCT AATCGTGATAGGGGTTTTTGCCTCCAACTG ACTCCTACATATTAGCATTAACAGTGTATG ATGCCTGTTACTAGCATTCA	NT_011512.7	74
mir-29b-3	AAGCTTCTTCAGGAAGCTGGTTTCATATGG TGGTTTAGATTTAAATAGTGATTGTCTAGC ACCATTTGAAATCAGTGTTCTTGGGGGAGA CCAGCTGCGCTGCACTACCA	NT_007933.10	75
mir-129-2	GGCATATTCTGCCCTTCGCGAATCTTTTTG CGGTCTGGGCTTGCTGTACATAACTCAATA GCCGGAAGCCCTTACCCCAAAAAGCATTTG CGGAGGGCGCACTCGTCGAG	NT_009237.13	76
mir-133b	CAGAAGAAAGATGCCCCCTGCTCTGGCTGG TCAAACGGAACCAAGTCCGTCTTCCTGAGA GGTTTGGTCCCCTTCAACCAGCTACAGCAG GGCTGGCAATGCCCAGTCCT	NT_007592.11	77
hypothetical miRNA-075	AGCGCAGCTTTAATTACTCATGCTGCTGGT TAAAATATTAATGGGGCACAGAGTGTTGCA TGCTCATTTCTGTTGATTTTTAATTAGCAG TAATTCATTTTGCACAAAGC	NT_006044.8	78
let-7d	AAAAAAATGGGTTCCTAGGAAGAGGTAGTA GGTTGCATAGTTTTAGGGCAGGGATTTTGC CCACAAGGAGGTAACTATACGACCTGCTGC CTTTCTTAGGGCCTTATTAT	NT_008476.13	79
mir-15b	AATCCTACATTTTTGAGGCCTTAAAGTACT GTAGCAGCACATCATGGTTTACATGCTACA GTCAAGATGCGAATCATTATTTGCTGCTCT AGAAATTTAAGGAAATTCAT	NT_005612.11	80
mir-29a-1	ACGACCTTCTGTGACCCCTTAGAGGATGAC TGATTTCTTTTGGTGTTCAGAGTCAATATA ATTTTCTAGCACCATCTGAAATCGGTTATA ATGATTGGGGAAGAGCACCA	NT_007933.10	81
hypothetical miRNA-079	CAAAGCTCTCCTGCCTGCTTCTGTGTGATA TGTTTGATATTGGGTTGTTTAATTAGGAAC CAACTAAATGTCAAACATATTCTTACAGCA GCAGGTGATTCAGCACCACC	NT_021907.13	82
mir-199b	CCAGAGGACACCTCCACTCCGTCTACCCAG TGTTTAGACTATCTGTTCAGGACTCCCAAA TTGTACAGTAGTCTGCACATTGGTTAGGCT GGGCTGGGTTAGACCCTCGG	NT_017568.11	83
mir-129-1	GGATGGCTGCTGTCTCCTTTGGATCTTTTT GCGGTCTGGGCTTGCTGTTCCTCTCAACAG TAGTCAGGAAGCCCTTACCCCAAAAAGTAT CTGCGGGAGGCCTTGTCCAC	NT_007933.10	84
let-7e	ACCTGCCGCGCCCCCCGGGCTGAGGTAGGA GGTTGTATAGTTGAGGAGGACACCCAAGGA GATCACTATACGGCCTCCTAGCTTTCCCCA GGCTGCGCCCTGCACGGGAC	NT_011109.13	85
hypothetical miRNA-083	TGGCAGGTTGTTTAGTTTTTTCGTTTGAAG GTTTTCATTAGTCTAATGAGGACTGTGCAA GGGCGAGCAGTCAGCACAATTTACATGGGG	NT_024524.11	86

	AAGCTATCATAATAAATGAA		
let-7c	AGCTGTGTGCATCCGGGTTGAGGTAGTAGG TTGTATGGTTTAGAGTTACACCCTGGGAGT TAACTGTACAACCTTCTAGCTTTCCTTGGA GCACACTTGAGCCGTCGAGG	NT_011512.7	87
mir-204	GGCTACAGTCTTTCTTCATGTGACTCGTGG ACTTCCCTTTGTCATCCTATGCCTGAGAAT ATATGAAGGAGGCTGGGAAGGCAAAGGGAC GTTCAATTGTCATCACTGGC	NT_008580.11	88
mir-145	CCACTCGCTCCCACCTTGTCCTCACGGTCC AGTTTTCCCAGGAATCCCTTAGATGCTAAG ATGGGGATTCCTGGAAATACTGTTCTTGAG GTCATGGTTTCACAGCTGGA	NT_006859.11	89
mir-124a-1	tccttcctCAGGAGAAAGGCCTCTCTCTCC GTGTTCACAGCGGACCTTGATTTAAATGTC CATACAATTAAGGCACGCGGTGAATGCCAA GAATGGGGCTGGCTGAGCAC	NT_019483.13	90
hypothetical miRNA-088	AGTCGCCAGTCACTTAAGCTGAGTGCATTG TGATTTCCAATAATTGAGGCAGTGGTTCTA AAAGCTGTCTACATTAATGAAAAGAGCAAT GTGGCCAGCTTGACTAAGCC	NT_011519.9	91
mir-213	TGAGTTTTGAGGTTGCTTCAGTGAACATTC AACGCTGTCGGTGAGTTTGGAATTAAAATC AAAACCATCGACCGTTGATTGTACCCTATG GCTAACCATCATCTACTCCA	NT_029862.8	92
hypothetical miRNA-090	CAGCGATACATTAATGCTCATTTGGCTCTG CAAATCTTACCGTTTGCTTAGGCCAAATGG CGCATCAATGACTATCGCTCTTACAAAGCT CTTGAATCAGTATTATGTAA	NT_006171.13	93
mir-20	TATCTGATGTGACAGCTTCTGTAGCACTAA AGTGCTTATAGTGCAGGTAGTGTTTAGTTA TCTACTGCATTATGAGCACTTAAAGTACTG CTAGCTGTAGAACTCCAGCT	NT_009952.11	94
mir-133a-1	CTAGCAGCACTACAATGCTTTGCTAGAGCT GGTAAAATGGAACCAAATCGCCTCTTCAAT GGATTTGGTCCCCTTCAACCAGCTGTAGCT ATGCATTGATTACTACGGGA	NT_011044.11	95
mir-138-2	GCGGAGTTCTGGTATCGTTGCTGCAGCTGG TGTTGTGAATCAGGCCGACGAGCAGCGCAT CCTCTTACCCGGCTATTTCACGACACCAGG GTTGCATCATACCCATCCTC	NT_010498.11	96
mir-98	CTGCTCATGCCAGGGTGAGGTAGTAAGTTG TATTGTTGTGGGGTAGGGATATTAGGCCCC AATTAGAAGATAACTATACAACTTACTACT TTCCCTGGTGTGTGGCATAT	NT_011799.10	97
mir-196-1	CTAGAGCTTGAATTGGAACTGCTGAGTGAA TTAGGTAGTTTCATGTTGTTGGGCCTGGGT TTCTGAACACAACAACATTAAACCACCCGA TTCACGGCAGTTACTGCTCC	NT_010783.11	98
mir-125b-1	AACATTGTTGCGCTCCTCTCAGTCCCTGAG ACCCTAACTTGTGATGTTTACCGTTTAAAT CCACGGGTTAGGCTCTTGGGAGCTGCGAGT CGTGCTTTTGCATCCTGGAA	NT_033899.3	99
mir-199a-2	AGGAAGCTTCTGGAGATCCTGCTCCGTCGC CCCAGTGTTCAGACTACCTGTTCAGGACAA TGCCGTTGTACAGTAGTCTGCACATTGGTT AGACTGGGCAAGGGAGAGCA	NT_029874.7	100
mir-29a-2	ACCTTCTGTGACCCCTTAGAGGATGACTGA TTTCTTTTGGTGTTCAGAGTCAATATAATT TTCTAGCACCATCTGAAATCGGTTATAATG ATTGGGGAAGAGCACCATGA	NT_007933.10	101

hypothetical miRNA-099	CTGGGGAGGTGAGCCTGAAAACAAAGGCAG ATAGAGAAGTCACAGCTCACTGGTGAGGGA GCTAGAGAGTTGTTTTCTTAATACCCTCTG CCTTTGAATCTGCCTAGATT	NT_016297.12	102
mir-181b	CCTGTGCAGAGATTATTTTTTAAAAGGTCA CAATCAACATTCATTGCTGTCGGTGGGTTG AACTGTGTGGACAAGCTCACTGAACAATGA ATGCAACTGTGGCCCCGCTT	NT_029862.8	103
hypothetical miRNA-101	GTATATTCAGGGACAGGCCATTGACAGTCA ATTAACAAGTTTGATTGGTATGTCAACTCA TTCTTTTGAATTGTTAATAGTATGTTAATA GCATTCGTTTCTTTGTGCAG	NT_030828.7	104
mir-141	CTGTCGGCCGGCCCTGGGTCCATCTTCCAG TACAGTGTTGGATGGTCTAATTGTGAAGCT CCTAACACTGTCTGGTAAAGATGGCTCCCG GGTGGGTTCTCTCGGCAGTA	NT_035206.1	105
mir-131-1	gccaggaggcggGGTTGGTTGTTATCTTTG GTTATCTAGCTGTATGAGTGGTGTGGAGTC TTCATAAAGCTAGATAACCGAAAGTAAAAA TAACCCCATACACTGCGCAG	NT_004858.13	106
mir-133a-2	TCGGATCTGGGAGCCAAATGCTTTGCTAGA GCTGGTAAAATGGAACCAAATCGACTGTCC AATGGATTTGGTCCCCTTCAACCAGCTGTA GCTGTGCATTGATGGCGCCG	NT_035608.1	107
hypothetical miRNA-105	CCGCCTCAGAGCCGCCCGCCGTTCCTTTTT CCTATGCATATACTTCTTTGAGGATCTGGC CTAAAGAGGTATAGGGCATGGGAAAACGGG GCGGTCGGGTCCTCCCCAGC	NT_017795.13	108
hypothetical miRNA-106	CGCCTCAGAGCCGCCCGCCGTTCCTTTTTC CTATGCATATACTTCTTTGAGGATCTGGCC TAAAGAGGTATAGGGCATGGGAAAACGGGG CGGTCGGGTCCTCCCCAGCG	NT_017795.13	109
hypothetical miRNA-107	CTATAATGCTTAGATTATCAATCATCTTGA CAGTTTATTGGCTTTATCACCACACATACC ATTAAAATGATGTCTGGCCTAGACTGTCAG GAGCAAACATTAAACAGACC	NT_008583.13	110
mir-1b	ACAGCTAACAACTTAGTAATACCTACTCAG AGTACATACTTCTTTATGTACCCATATGAA CATACAATGCTATGGAATGTAAAGAAGTAT GTATTTTTGGTAGGCAATAA	NT_011044.11	111
mir-18	ATGTTGAGTGCTTTTTGTTCTAAGGTGCAT CTAGTGCAGATAGTGAAGTAGATTAGCATC TACTGCCCTAAGTGCTCCTTCTGGCATAAG AAGTTATGTATTCATCCAAT	NT_009952.11	112
mir-220	GACAGTGTGGCATTGTAGGGCTCCACACCG TATCTGACACTTTGGGCGAGGGCACCATGC TGAAGGTGTTCATGATGCGGTCTGGGAACT CCTCACGGATCTTACTGATG	NT_011588.11	113
hypothetical miRNA-111	CTCTGGCCTCCGCTTCCTCCTCCTCCGACT CGGACACCGGCGGAGCCTCCCCGCCCCCGC GGAAGAAACCCCGAGCCTCGGCGGCGGAGG GAGTAGGAGAGCCCGGGGCT	NT_004525.13	114
mir-7-3	AGATTAGAGTGGCTGTGGTCTAGTGCTGTG TGGAAGACTAGTGATTTTGTTGTTCTGATG TACTACGACAACAAGTCACAGCCGGCCTCA TAGCGCAGACTCCCTTCGAC	NT_011255.11	115
mir-218-2	GACCAGTCGCTGCGGGGCTTTCCTTTGTGC TTGATCTAACCATGTGGTGGAACGATGGAA ACGGAACATGGTTCTGTCAAGCACCGCGGA AAGCACCGTGCTCTCCTGCA	NT_023132.10	116
mir-24-2	GCCTGGCCTCCCTGGGCTCTGCCTCCCGTG	NT_031915.4	117

	CCTACTGAGCTGAAACACAGTTGGTTTGTG TACACTGGCTCAGTTCAGCAGGAACAGGGG TCAAGCCCCCTTGGAGCCTG		
mir-24-1	CTGTCGATTGGACCCGCCCTCCGGTGCCTA CTGAGCTGATATCAGTTCTCATTTTACACA CTGGCTCAGTTCAGCAGGAACAGGAGTCGA GCCCTTGAGCAAAAAGCCTT	NT_008476.13	118
mir-103-2	CTCAGCAGAGCTGCGTCTTTGTGCTTTCAG CTTCTTTACAGTGCTGCCTTGTAGCATTCA GGTCAAGCAGCATTGTACAGGGCTATGAAA GAACCAAGAATGGGCTGCCC	NT_011387.8	119
mir-211	TCACCTGGCCATGTGACTTGTGGGCTTCCC TTTGTCATCCTTCGCCTAGGGCTCTGAGCA GGGCAGGGACAGCAAAGGGGTGCTCAGTTG TCACTTCCCACAGCACGGAG	NT_010363.13	120
mir-101-3	AGGTAGATATGAGACTGAACTGTCCTTTTT CGGTTATCATGGTACCGATGCTGTATATCT GAAAGGTACAGTACTGTGATAACTGAAGAA TGGTGGTGCCATCACATTGA	NT_008413.13	121
mir-30b	CCAAGTTTCAGTTCATGTAAACATCCTACA CTCAGCTGTAATACATGGATTGGCTGGGAG GTGGATGTTTACTTCAGCTGACTTGGAATG TCAACCAATTAACATTGATA	NT_028251.8	122
hypothetical miRNA-120	GGCTTCTTCCAGTCATCCTGAGGTAGATAT CATCCAGGAATGCTGAGGCCTTATGGCTTA CAGCAATCCAGTAATGATATAAAAGGTGAT TGGAGGTTAGATTTACATTG	NT_009952.11	123
let-7a-4	TGTGACTGCATGCTCCCAGGTTGAGGTAGT AGGTTGTATAGTTTAGAATTACATCAAGGG AGATAACTGTACAGCCTCCTAGCTTTCCTT GGGTCTTGCACTAAACAACA	NT_033899.3	124
mir-10a	GATCTGTCTGTCTTCTGTATATACCCTGTA GATCCGAATTTGTGTAAGGAATTTTGTGGT CACAAATTCGTATCTAGGGGAATATGTAGT TGACATAAACACTCCGCTCT	NT_010783.11	125
mir-19a	TTGCAGTCCTCTGTTAGTTTTGCATAGTTG CACTACAAGAAGAATGTAGTTGTGCAAATC TATGCAAAACTGATGGTGGCCTGCTATTTC CTTCAAATGAATGATTTTTA	NT_009952.11	126
let-7f-2	ACACTGGTGCTCTGTGGGATGAGGTAGTAG ATTGTATAGTTTTAGGGTCATACCCCATCT TGGAGATAACTATACAGTCTACTGTCTTTC CCACGGTGGTACACTTCTTC	NT_011799.10	127
mir-15a-1	CGCGAATGTGTGTTTAAAAAAAATAAAACC TTGGAGTAAAGTAGCAGCACATAATGGTTT GTGGATTTTGAAAAGGTGCAGGCCATATTG TGCTGCCTCAAAAATACAAG	NT_010393.11	128
mir-108-2	CCGAGGAATACTGCAAGAGCAATAAGGATT TTTAGGGGCATTATGATAGTGGAATGGAAA CACATCTGCCCCCAAAAGTCCCTCATTTTC CCTGCGGTAACGAACCAGCT	NT_034392.2	129
mir-137	cTTGGTCCTCTGACTCTCTTCGGTGACGGG TATTCTTGGGTGGATAATACGGATTACGTT GTTATTGCTTAAGAATACGCGTAGTCGAGG AGAGTACCAGCGGCAGGGGG	NT_033951.3	130
mir-219	CCGCCCCGGGCCGCGGCTCCTGATTGTCCA AACGCAATTCTCGAGTCTATGGCTCCGGCC GAGAGTTGAGTCTGGACGTCCCGAGCCGCC GCCCCCAAACCTCGAGCGGG	NT_007592.11	131
mir-148b	CATTTCCAAGCACGATTAGCATTTGAGGTG AAGTTCTGTTATACACTCAGGCTGTGGCTC	NT_009458.12	132

	TCTGAAAGTCAGTGCATCACAGAACTTTGT CTCGAAAGCTTTCTAGCAGC		
mir-130b	GGGGAGGCACTGGCAGGCCTGCCCGACACT CTTTCCCTGTTGCACTACTATAGGCCGCTG GGAAGCAGTGCAATGATGAAAGGGCATCGG TCAGGTCCAGCCTGCTACCC	NT_011520.8	133
mir-19b-1	CCTGTTACTGAACACTGTTCTATGGTTAGT TTTGCAGGTTTGCATCCAGCTGTGTGATAT TCTGCTGTGCAAATCCATGCAAAACTGACT GTGGTAGTGAAAAGTCTGTA	NT_009952.11	134
let-7a-2	TTGTGACTGCATGCTCCCAGGTTGAGGTAG TAGGTTGTATAGTTTAGAATTACATCAAGG GAGATAACTGTACAGCCTCCTAGCTTTCCT TGGGTCTTGCACTAAACAAC	NT_033899.3	135
mir-216	GATGGCTGTGAGTTGGCTTAATCTCAGCTG GCAACTGTGAGATGTTCATACAATCCCTCA CAGTGGTCTCTGGGATTATGCTAAACAGAG CAATTTCCTAGCCCTCACGA	NT_005375.11	136
mir-100-1	AAAGAGAGAAGATATTGAGGCCTGTTGCCA CAAACCCGTAGATCCGAACTTGTGGTATTA GTCCGCACAAGCTTGTATCTATAGGTATGT GTCTGTTAGGCAATCTCACG	NT_033899.3	137
mir-100-2	AAAGAGAGAAGATATTGAGGCCTGTTGCCA CAAACCCGTAGATCCGAACTTGTGGTATTA GTCCGCACAAGCTTGTATCTATAGGTATGT GTCTGTTAGGCAATCTCACG	NT_033899.3	137
mir-187	GGTCGGGCTCACCATGACACAGTGTGAGAC CTCGGGCTACAACACAGGACCCGGGGCGCT GCTCTGACCCCTCGTGTCTTGTGTTGCAGC CGGAGGGACGCAGGTCCGCA	NT_010966.11	139
hypothetical miRNA-137	GTTCAACATAAGCAAACAGATTGTAAACTG GCTGATAATTTTTGTACTGACAATGTCATT TACAGCTGTCAGCCTTTCGTCTGCCTTGTT TGCTTTATTCAAATATGAAC	NT_011387.8	140
hypothetical miRNA-138	CCCTCCAATGTCTGATTAATCAAGCCTGCA AACAGCTTATTTCTTTTAGCCTGCATGCAA GTATGAAAATGAGATTCTGGGAGCCGAACA TGGTGCAGATTTGTTCATTC	NT_008902.13	141
hypothetical miRNA-139	CCCCTCCAATGTCTGATTAATCAAGCCTGC AAACAGCTTATTTCTTTTAGCCTGCATGCA AGTATGAAAATGAGATTCTGGGAGCCGAAC ATGGTGCAGATTTGTTCATT	NT_008902.13	142
mir-124a-3	CCCGCCCCAGCCCTGAGGGCCCCTCTGCGT GTTCACAGCGGACCTTGATTTAATGTCTAT ACAATTAAGGCACGCGGTGAATGCCAAGAG AGGCGCCTCCGCCGCTCCTT	NT_011333.5	143
mir-7-2	CTGGATACAGAGTGGACCGGCTGGCCCCAT CTGGAAGACTAGTGATTTTGTTGTTGTCTT ACTGCGCTCAACAACAAATCCCAGTCTACC TAATGGTGCCAGCCATCGCA	NT_033276.3	144
hypothetical miRNA-142	gggGTGAATTTATTTCTTACAGAACCGCCC TGATTCAGATGGTGCAAGCCTCGCAGGCCA GAAACATCTTCCTGACGCTGCTCCCCACCT TCTGCCCTCTCTTTCCCAGC	NT_033317.3	145
hypothetical miRNA-143	GCTGATGAAAATAGGGCAGTGGTTTAAATA GATTTGCAAGCAATTTACCTTTTCACAATG TTGGCAATCTGATGCAATTTGCTTGCAATT TTGTCTGCTTTCAGTAGCAC	NT_007819.11	146
hypothetical miRNA-144	CACGCATGAGCGGACGCTAACCCCCTCCCC AGCCACAAAGAGTCTACATGTCTAGGGTCT AGACATGTTCAGCTTTGTGGACCTCCGGCT	NT_010783.11	147

	CCTGCTCCTCTTAGCGGCCA		
mir-210	ACCCGGCAGTGCCTCCAGGCGCAGGGCAGC CCCTGCCCACCGCACACTGCGCTGCCCCAG ACCCACTGTGCGTGTGACAGCGGCTGATCT GTGCCTGGGCAGCGCGACCC	NT_035113.2	148
mir-215	ATCATTCAGAAATGGTATACAGGAAAATGA CCTATGAATTGACAGACAATATAGCTGAGT TTGTCTGTCATTTCTTTAGGCCAATATTCT GTATGACTGTGCTACTTCAA	NT_021953.13	149
mir-223	CCTGGCCTCCTGCAGTGCCACGCTCCGTGT ATTTGACAAGCTGAGTTGGACACTCCATGT GGTAGAGTGTCAGTTTGTCAAATACCCCAA GTGCGGCACATGCTTACCAG	NT_011669.11	150
mir-131-3	CACGGCGCGGCAGCGGCACTGGCTAAGGGA GGCCCGTTTCTCTCTTTGGTTATCTAGCTG TATGAGTGCCACAGAGCCGTCATAAAGCTA gataaccgaaagtagaaatg	NT_033276.3	151
mir-199a-1	TGGATAGCCGGCCCCGCCAACCCAGTGTTC AGACTACCTGTTCAGGAGGCTCTCAATGTG TACAGTAGTCTGCACATTGGTTAGGCTGGG CTTGGGTGAGCGGCTCGTCG	NT_011176.13	152
mir-30c	CCTAGAGAGCACTGAGCGACAGATACTGTA AACATCCTACACTCTCAGCTGTGGAAAGTA AGAAAGCTGGGAGAAGGCTGTTTACTCTTT CTGCCTTGGAAGTCAACTAA	NT_007299.11	153
mir-101-1	AGGCTGCCCTGGCTCAGTTATCACAGTGCT GATGCTGTCTATTCTAAAGGTACAGTACTG TGATAACTGAAGGATGGCAGCCATCTTACC TTCCATCAGAGGAGCCTCAC	NT_029865.8	154
mir-101-2	AGGCTGCCCTGGCTCAGTTATCACAGTGCT GATGCTGTCTATTCTAAAGGTACAGTACTG TGATAACTGAAGGATGGCAGCCATCTTACC TTCCATCAGAGGAGCCTCAC	NT_029865.8	154
hypothetical miRNA-153	TGTTGAATGCAAGCAGATGCTGATAATATC AGAAGTCACAGCATAATTTTTTTTGATCAA AGGGCTCAAGTGAGCCTGATGAAGCATGCA TCTTGCTCGTCTTTGATAAA	NT_005332.11	156
hypothetical miRNA-154	CCTGCAGTGATGCTTCATGAGCAAATCACA TGATGTCAGAATGGTATGGTTTCGATTTAA TCAAGAAAGAGATTAAAGTGGATGTGTGTT ATTTTCAACTTCGCAGCAGC	NT_030828.7	157
mir-26b	CGCCCCACCCTGCCCGGGACCCAGTTCAAG TAATTCAGGATAGGTTGTGTGCTGTCCAGC CTGTTCTCCATTACTTGGCTCGGGGACCGG TGCCCTGCAGCCTTGGGGTG	NT_005403.11	158
hypothetical miRNA-156	TGCGTTTACATAACACCAGGCGTGTGGGAG CTGGAGGAAGAGGTTGCGAATGTAGGAGAG ATAAGGCTCCTGCTTTCCCTCCTTCCTTCT TGGTGGTACCAGGCTTGACA	NT_029289.7	159
mir-152	GGCCCGCTGTCCCCCCCGGCCCAGGTTCTG TGATACACTCCGACTCGGGCTCTGGAGCAG TCAGTGCATGACAGAACTTGGGCCCGGAAG GACCTTCTGCACCCAACGGG	NT_010783.11	160
mir-135-1	CAGCCCCAGGCCTCGCTGTTCTCTATGGCT TTTTATTCCTATGTGATTCTACTGCTCACT CATATAGGGATTGGAGCCGTGGCGCACGGC GGGGACAGCCAGCGGAGGGT	NT_005986.13	161
mir-135-2	ACCAAGATAAATTCACTCTAGTGCTTTATG GCTTTTTATTCCTATGTGATAGTAATAAAG TCTCATGTAGGGATGGAAGCCATGAAATAC ATTGTGAAAAATCATCAACT	NT_009681.13	162

mir-217	AGTATAATTATTACATAGTTTTTGATGTCG CAGATACTGCATCAGGAACTGATTGGATAA GAATCAGTCACCATCAGTTCCTAATGCATT GCCTTCAGCATCTAAACAAG	NT_005375.11	163
hypothetical miRNA-161	CTTGGCCATAAACTTGTAGTCATCCTCTAT CCAATCATATTGTCTTGAGTAATTAAAATG ATTAGCTTAATTAGCTTAATTAACTAAATT TGACTACAGGACATGGCCAT	NT_004658.12	164
mir-15a-2	GGCGCGAATGTGTGTTTAAAAAAAATAAAA CCTTGGAGTAAAGTAGCAGCACATAATGGT TTGTGGATTTTGAAAAGGTGCAGGCCATAT TGTGCTGCCTCAAAAATACA	NT_033922.3	165
let-7g	TTTGCCTGATTCCAGGCTGAGGTAGTAGTT TGTACAGTTTGAGGGTCTATGATACCACCC GGTACAGGAGATAACTGTACAGGCCACTGC CTTGCCAGGAACAGCGCGCC	NT_005986.13	166
hypothetical miRNA-164	AATTGTCCTTGGTTTTACAATGATAAATGA AAAACATTAAAATTCTCCAACTGAACAGGT ATGCAAGGATTTTTATGTTTTGTTGTTGTT GTTAAAACAGTGAGAGCAAA	NT_010783.11	167
mir-33b	GGGGGCCGAGAGAGGCGGGCGGCCCCGCGG TGCATTGCTGTTGCATTGCACGTGTGTGAG GCGGGTGCAGTGCCTCGGCAGTGCAGCCCG GAGCCGGCCCCTGGCACCAC	NT_030843.4	168
hypothetical miRNA-166	GCAGTGGCGCTCAATGCTGTGCACTTCCAG TTGCAGCACCTGTAAGGTTTGTTAAAGGTA AAGGCAGGTCGGGAAAAGGTGCTTCGAGGA AGAGGCCTGGGAGGGGGCGA	NT_011588.11	169
mir-16-2	GCAATGTCAGCAGTGCCTTAGCAGCACGTA AATATTGGCGTTAAGATTCTAAAATTATCT CCAGTATTAACTGTGCTGCTGAAGTAAGGT TGACCATACTCTACAGTTGT	NT_033922.3	170
hypothetical miRNA-168	ATGGACAAGATCTATGACGGCCAAGTGGAG GTGACTGGCGATAAATACAATGTGGAAAGT ACTGATGGTCAGCCAGGTGCCTTCACCTGC TGTATGGATGCAGGTCTTGC	NT_011520.8	171
hypothetical miRNA-169	CACTGGAGGCTGTTCTATAAATGATCATTG AAGGGCTGCAAGCTAGCCTATAATTACAGG AAAGAAAGTGGCAGCTCTGGCATTTCATAA CTATGTGTCCTCGAAAAGTG	NT_007933.10	172
hypothetical miRNA-170	GAATGTATGATCTTGCTCTAACACTTGGCC AGACCTGTGTCACCCACTGCTAGTGCCTGA AGTCGACAGACAATTCTGCCAAGGTAACCG AGAATCATTAAGCATCCTGC	NT_005151.11	173
hypothetical miRNA-171	CACCCTGTCTGACAAGTATGTTTTATCGTT TCAAGAAATGCGGTTAACCTCGCAGTACTA AAACTGAATGAACAAGGCCTGTTGGACAAA TTGAAAAACAAATGGTGGTA	NT_006171.13	174
hypothetical miRNA-172	TGTTTTTTTGAGTACATGTGTATAAATAGA GGTGGCTTCCTGTCAGTTTGGTATTATTGA TATGATCCAACTGCAAGAAGTTACTGCAAC ACTTTGCATCTTAAAGGTCC	NT_037752.1	175
hypothetical miRNA-173	TAGTTCAGCACTCTTACCTCTTATTGGTGT ACCACCTGGGTGGATAATATGAATGCAAAT AAGATTAGAAAGAAGAAGCATTAGTACGAG AAGAAGGAGGCTAGGGCTGG	NT_008413.13	176
mir-182	GAGCTGCTTGCCTCCCCCCGTTTTTGGCAA TGGTAGAACTCACACTGGTGAGGTAACAGG ATCCGGTGGTTCTAGACTTGCCAACTATGG GGCGAGGACTCAGCCGGCAC	NT_007933.10	177
hypothetical	CTTGCCAGAAACATCAGTGACATGGACAAA	NT_006258.12	178

miRNA-175	GGTGTCATTGAAGGAGACAAAGATGTGGCA GGCACCAAATACATTCTCTCCTTCAACCAC CTGAGGTCCGAGGCTGATGA		
hypothetical miRNA-176	TGGAAGGAAAATAGGAGTTTGATATGACAT ATTGTGTGTCTCAGCAAGACTCATAAATAA TTTTGACAAGTTTTTGTATGCATGGGAAAG TCCTTGATTCAGCCTCCCAT	NT_025004.11	179
hypothetical miRNA-177	GGGAACCAGCGCTTTCAGTAAGAGAGTGGT ACCACGTGTCTTCAAAATGAAACGTTTCTT GGAGACAAACATGCTACTCTCACTGAGTAC ACAAGCTTCCTGGTTGTCAG	NT_023098.7	180
hypothetical miRNA-178	CCAGTTTCCATCTGTCATGATAGCCTATCT CCGAACCTTCAATCTGTCAAAAGCTCGCTG CCTGGCTGAAGGCTCCAGGAGATTTGGTGC ACTAAACACATTTGACAACA	NT_037537.1	181
hypothetical miRNA-179	AATGCCAGTGAGTTTGAAAGGCACTTTGTC CAATTAGAAGTGTGGAGAAATATTCATCCT GTCCATGACAAAGATGAAGTGCTTCTTTCA AAAGCGGCGGTGGCAGGCTG	NT_010194.13	182
hypothetical miRNA-180	AGCACTTCTACATGATCCTATGACTCTTGA TATGGACGCAGTCCTGTCAGACTTTGTTCG GTCCACGGGGGCAGAACCTGGTCTGGCCAG AGACCTGCTGGAAGGTAAGC	NT_010363.13	183
hypothetical miRNA-181	TTGTGCACCTCACCTGCTCTGGAAGTAGTT TGCTAGCTCTGATGCTTCATGGTTCAGACT CCTCAGGTGCACGATTAAATTTCCAGAGTT GGTGAACATGGCGCCACATG	NT_033899.3	184
mir-148a	GGAGGAAGACAGCACGTTTGGTCTTTTGAG GCAAAGTTCTGAGACACTCCGACTCTGAGT ATGATAGAAGTCAGTGCACTACAGAACTTT GTCTCTAGAGGCTGTGGTCG	NT_007819.11	185
hypothetical miRNA-183	ACTCCAGGTGAAACACTGCTGAGTCCTTTG GTGATGTGTGGTCCCCATGGCCTCAAGTTC CTGAAGCCTGTGGAGCTGCGCTTACCACAC TGTGCGTCCATGACTCCTGA	NT_010363.13	186
mir-23a	CTCACCCCTGTGCCACGGCCGGCTGGGGTT CCTGGGGATGGGATTTGCTTCCTGTCACAA ATCACATTGCCAGGGATTTCCAACCGACCC TGAGCTCTGCCACCGAGGAT	NT_031915.4	187
hypothetical miRNA-185	ACACAAAACATGAACTGTGTACTCATTGTC TTCGCTGCACAGCTTGGCATTGGGGTTGGT GACTCTGATGGCCAGCTGAGCAGCTCTTTC CACAATGGCTTTGTGGTCCT	NT_007592.11	188
hypothetical miRNA-186	ATATGGGAACCAGTGCTTGCAGAAAGAGGG TAGTTCCACATGTCTGCAAAACGAGACATC TCTTGAAGACAAACATGCTACTCTCACTGC GTACATAAGCTTCCTATTTG	NT_008705.13	189
mir-181c	CGGAAAATTTGCCAAGGGTTTGGGGGAACA TTCAACCTGTCGGTGAGTTTGGGCAGCTCA GGCAAACCATCGACCGTTGAGTGGACCCTG AGGCCTGGAATTGCCATCCT	NT_031915.4	190
hypothetical miRNA-188	AGAATGGTATCATAGGACAGTGTGATGGAA TTTTTCTTTCTCTGTCATCATTAAGGGGGT TCCCCCTATGGTGAGGGGAATGAAAAGTAC GATTTAATGTTCTCTGGAGA	NT_023148.9	191

Example 4: miRNAs within pri-miRNAs

miRNAs found within the pri-miRNA structures disclosed above were used in certain embodiments of the present invention. These miRNAs represent target nucleic acids to which the oligomeric compounds of the present invention were designed. The oligomeric compounds of the present invention can also be designed to mimic the miRNA while incorporating certain chemical modifications that alter one or more properties of the mimic, thereby creating a construct with superior properties over the endogenous miRNA. The miRNA target sequences are shown in Table 2.

Table 2

miRNAs found within pri-miRNAs

Pri-miRNA	miRNA sequence (DNA form; where T replaces U in RNA)	SEQ ID NO
mir-140	AGTGGTTTTACCCTATGGTAG	192
mir-30a	CTTTCAGTCGGATGTTTGCAGC	193
mir-34	TGGCAGTGTCTTAGCTGGTTGT	194
mir-29b-1	TAGCACCATTTGAAATCAGTGTT	195
mir-29b-2	TAGCACCATTTGAAATCAGTGTT	195
mir-16-3	TAGCAGCACGTAAATATTGGCG	196
mir-203	GTGAAATGTTTAGGACCACTAG	197
mir-7-1	TGGAAGACTAGTGATTTTGTT	198
mir-10b	TACCCTGTAGAACCGAATTTGT	199
mir-128a	TCACAGTGAACCGGTCTCTTTT	200
mir-153-1	TTGCATAGTCACAAAAGTGA	201
mir-153-2	TTGCATAGTCACAAAAGTGA	201
mir-27b	TTCACAGTGGCTAAGTTCTG	202
mir-96	TTTGGCACTAGCACATTTTTGC	203
mir-17as/mir-91	CAAAGTGCTTACAGTGCAGGTAGT	204
mir-123/mir-126as	CATTATTACTTTTGGTACGCG	205
mir-132	TAACAGTCTACAGCCATGGTCGC	206
mir-108-1	ATAAGGATTTTTAGGGGCATT	207
mir-23b	ATCACATTGCCAGGGATTACCAC	208
let-7i	TGAGGTAGTAGTTTGTGCT	209
mir-212	TAACAGTCTCCAGTCACGGCC	210
mir-131-2	TAAAGCTAGATAACCGAAAGT	211
let-7b	TGAGGTAGTAGGTTGTGTGGTT	212
mir-1d	TGGAATGTAAAGAAGTATGTAT	213
mir-122a	TGGAGTGTGACAATGGTGTTTGT	214
mir-22	AAGCTGCCAGTTGAAGAACTGT	215
mir-92-1	TATTGCACTTGTCCCGGCCTGT	216
mir-142	CATAAAGTAGAAAGCACTAC	217
mir-183	TATGGCACTGGTAGAATTCACTG	218
mir-214	ACAGCAGGCACAGACAGGCAG	219
mir-143	TGAGATGAAGCACTGTAGCTCA	220
mir-192-1	CTGACCTATGAATTGACAGCC	221
mir-192-2	CTGACCTATGAATTGACAGCC	221
mir-192-3	CTGACCTATGAATTGACAGCC	221
let-7a-3	TGAGGTAGTAGGTTGTATAGTT	222
mir-181a	AACATTCAACGCTGTCGGTGAGT	223
let-7a-1	TGAGGTAGTAGGTTGTATAGTT	222
mir-205	TCCTTCATTCCACCGGAGTCTG	224
mir-103-1	AGCAGCATTGTACAGGGCTATGA	225

mir-26a	TTCAAGTAATCCAGGATAGGCT	226
mir-33a	GTGCATTGTAGTTGCATTG	227
mir-196-2	TAGGTAGTTTCATGTTGTTGGG	228
mir-107	AGCAGCATTGTACAGGGCTATCA	229
mir-106	AAAAGTGCTTACAGTGCAGGTAGC	230
let-7f-1	TGAGGTAGTAGATTGTATAGTT	231
mir-29c	CTAGCACCATTTGAAATCGGTT	232
mir-130a	CAGTGCAATGTTAAAAGGGC	233
mir-218-1	TTGTGCTTGATCTAACCATGT	234
mir-124a-2	TTAAGGCACGCGGTGAATGCCA	235
mir-21	TAGCTTATCAGACTGATGTTGA	236
mir-16-1	TAGCAGCACGTAAATATTGGCG	196
mir-144	TACAGTATAGATGATGTACTAG	237
mir-221	AGCTACATTGTCTGCTGGGTTTC	238
mir-222	AGCTACATCTGGCTACTGGGTCTC	239
mir-30d	TGTAAACATCCCCGACTGGAAG	240
mir-19b-2	TGTGCAAATCCATGCAAAACTGA	241
mir-128b	TCACAGTGAACCGGTCTCTTTC	242
mir-29b-3	TAGCACCATTTGAAATCAGTGTT	195
mir-129-2	CTTTTTGCGGTCTGGGCTTGC	243
mir-133b	TTGGTCCCCTTCAACCAGCTA	244
let-7d	AGAGGTAGTAGGTTGCATAGT	245
mir-15b	TAGCAGCACATCATGGTTTACA	246
mir-29a-1	CTAGCACCATCTGAAATCGGTT	247
mir-199b	CCCAGTGTTTAGACTATCTGTTC	248
mir-129-1	CTTTTTGCGGTCTGGGCTTGC	243
let-7e	TGAGGTAGGAGGTTGTATAGT	249
let-7c	TGAGGTAGTAGGTTGTATGGTT	250
mir-204	TTCCCTTTGTCATCCTATGCCT	251
mir-145	GTCCAGTTTTCCCAGGAATCCCTT	252
mir-124a-1	TTAAGGCACGCGGTGAATGCCA	235
mir-213	ACCATCGACCGTTGATTGTACC	253
mir-20	TAAAGTGCTTATAGTGCAGGTAG	254
mir-133a-1	TTGGTCCCCTTCAACCAGCTGT	255
mir-138-2	AGCTGGTGTTGTGAATC	256
mir-98	TGAGGTAGTAAGTTGTATTGTT	257
mir-196-1	TAGGTAGTTTCATGTTGTTGGG	228
mir-125b-1	TCCCTGAGACCCTAACTTGTGA	258
mir-199a-2	CCCAGTGTTCAGACTACCTGTTC	259
mir-29a-2	CTAGCACCATCTGAAATCGGTT	247
mir-181b	AACATTCATTGCTGTCGGTGGGTT	260
mir-141	AACACTGTCTGGTAAAGATGG	261
mir-131-1	TAAAGCTAGATAACCGAAAGT	211
mir-133a-2	TTGGTCCCCTTCAACCAGCTGT	255
mir-1b	TGGAATGTAAAGAAGTATGTAT	213
mir-18	TAAGGTGCATCTAGTGCAGATA	262
mir-220	CCACACCGTATCTGACACTTT	263
mir-7-3	TGGAAGACTAGTGATTTTGTT	198
mir-218-2	TTGTGCTTGATCTAACCATGT	234
mir-24-2	TGGCTCAGTTCAGCAGGAACAG	264
mir-24-1	TGGCTCAGTTCAGCAGGAACAG	264
mir-103-2	AGCAGCATTGTACAGGGCTATGA	225
mir-211	TTCCCTTTGTCATCCTTCGCCT	264
mir-101-3	TACAGTACTGTGATAACTGA	265
mir-30b	TGTAAACATCCTACACTCAGC	266
let-7a-4	TGAGGTAGTAGGTTGTATAGTT	222
mir-10a	TACCCTGTAGATCCGAATTTGTG	267
mir-19a	TGTGCAAATCTATGCAAAACTGA	268
let-7f-2	TGAGGTAGTAGATTGTATAGTT	231

mir-15a-1	TAGCAGCACATAATGGTTTGTG	269
mir-108-2	ATAAGGATTTTTAGGGGCATT	207
mir-137	TATTGCTTAAGAATACGCGTAG	270
mir-219	TGATTGTCCAAACGCAATTCT	271
mir-148b	TCAGTGCATCACAGAACTTTGT	272
mir-130b	CAGTGCAATGATGAAAGGGC	273
mir-19b-1	TGTGCAAATCCATGCAAAACTGA	241
let-7a-2	TGAGGTAGTAGGTTGTATAGTT	222
mir-216	TAATCTCAGCTGGCAACTGTG	274
mir-100-1	AACCCGTAGATCCGAACTTGTG	275
mir-100-2	AACCCGTAGATCCGAACTTGTG	275
mir-187	TCGTGTCTTGTGTTGCAGCCGG	276
mir-124a-3	TTAAGGCACGCGGTGAATGCCA	235
mir-7-2	TGGAAGACTAGTGATTTTGTT	198
mir-210	CTGTGCGTGTGACAGCGGCTG	277
mir-215	ATGACCTATGAATTGACAGAC	278
mir-223	TGTCAGTTTGTCAAATACCCC	279
mir-131-3	TAAAGCTAGATAACCGAAAGT	211
mir-199a-1	CCCAGTGTTCAGACTACCTGTTC	259
mir-30c	TGTAAACATCCTACACTCTCAGC	280
mir-101-1	TACAGTACTGTGATAACTGA	265
mir-101-2	TACAGTACTGTGATAACTGA	265
mir-26b	TTCAAGTAATTCAGGATAGGTT	281
mir-152	TCAGTGCATGACAGAACTTGG	282
mir-135-1	TATGGCTTTTTATTCCTATGTGAT	283
mir-135-2	TATGGCTTTTTATTCCTATGTGAT	283
mir-217	TACTGCATCAGGAACTGATTGGAT	284
mir-15a-2	TAGCAGCACATAATGGTTTGTG	269
let-7g	TGAGGTAGTAGTTTGTACAGT	285
mir-33b	GTGCATTGCTGTTGCATTG	286
mir-16-2	TAGCAGCACGTAAATATTGGCG	196
mir-182	TTTGGCAATGGTAGAACTCACA	287
mir-148a	TCAGTGCACTACAGAACTTTGT	288
mir-23a	ATCACATTGCCAGGGATTTCC	289
mir-181c	AACATTCAACCTGTCGGTGAGT	290

Example 5: Uniform 2'-MOE phosphorothioate (PS) oligomeric compounds targeting miRNAs

In accordance with the present invention, a series of oligomeric compounds were designed and synthesized to target miRNA sequences disclosed by Lim et al. *Science,* **2003**, 299, 1540. The compounds are shown in Table 3. "Pri-miRNA" indicates the particular pri-miRNA which contains the miRNA that the oligomeric compound was designed to target. All compounds in Table 3 are composed of 2'-methoxyethoxy (2'-MOE) nucleotides throughout and the internucleoside (backbone) linkages are phosphorothioate (P=S) throughout. All cytidine residues are 5-methylcytidines. The compounds can be analyzed for their effect on miRNA, pre-miRNA or pri-miRNA levels by quantitative real-time PCR as described, *supra*, or they can be used in other assays to investigate the role of miRNAs or the function of targets downstream of miRNAs.

Table 3

Uniform 2'-MOE PS Compounds targeting miRNAs

ISIS Number	SEQ ID NO	Sequence	Pri-miRNA
327873	291	CTACCATAGGGTAAAACCACT	mir-140
327874	292	GCTGCAAACATCCGACTGAAAG	mir-30a
327875	293	ACAACCAGCTAAGACACTGCCA	mir-34
327876	294	AACACTGATTTCAAATGGTGCTA	mir-29b-1
327877	295	CGCCAATATTTACGTGCTGCTA	mir-16-3
327878	296	CTAGTGGTCCTAAACATTTCAC	mir-203
327879	297	AACAAAATCACTAGTCTTCCA	mir-7-1
327880	298	ACAAATTCGGTTCTACAGGGTA	mir-10b
327881	299	AAAAGAGACCGGTTCACTGTGA	mir-128a
327882	300	TCACTTTTGTGACTATGCAA	mir-153-1
327883	301	CAGAACTTAGCCACTGTGAA	mir-27b
327884	302	GCAAAAATGTGCTAGTGCCAAA	mir-96
327885	303	ACTACCTGCACTGTAAGCACTTTG	mir-17as/mir-91
327886	304	CGCGTACCAAAAGTAATAATG	mir-123/mir-126as
327887	305	GCGACCATGGCTGTAGACTGTTA	mir-132
327888	306	AATGCCCCTAAAAATCCTTAT	mir-108-1
327889	307	GTGGTAATCCCTGGCAATGTGAT	mir-23b
327890	308	AGCACAAACTACTACCTCA	let-7i
327891	309	GGCCGTGACTGGAGACTGTTA	mir-212
327892	310	ACTTTCGGTTATCTAGCTTTA	mir-131-2/mir-9
327893	311	AACCACACAACCTACTACCTCA	let-7b
327894	312	ATACATACTTCTTTACATTCCA	mir-1d
327895	313	ACAAACACCATTGTCACACTCCA	mir-122a
327896	314	ACAGTTCTTCAACTGGCAGCTT	mir-22
327897	315	ACAGGCCGGGACAAGTGCAATA	mir-92-1
327898	316	GTAGTGCTTTCTACTTTATG	mir-142
327899	317	CAGTGAATTCTACCAGTGCCATA	mir-183
327900	318	CTGCCTGTCTGTGCCTGCTGT	mir-214
327901	319	TGAGCTACAGTGCTTCATCTCA	mir-143
327902	320	GGCTGTCAATTCATAGGTCAG	mir-192-1
327903	321	AACTATACAACCTACTACCTCA	let-7a-3
327904	322	ACTCACCGACAGCGTTGAATGTT	mir-181a
327905	323	CAGACTCCGGTGGAATGAAGGA	mir-205
327906	324	TCATAGCCCTGTACAATGCTGCT	mir-103-1
327907	325	AGCCTATCCTGGATTACTTGAA	mir-26a
327908	326	CAATGCAACTACAATGCAC	mir-33a
327909	327	CCCAACAACATGAAACTACCTA	mir-196-2
327910	328	TGATAGCCCTGTACAATGCTGCT	mir-107
327911	329	GCTACCTGCACTGTAAGCACTTTT	mir-106
327912	330	AACTATACAATCTACTACCTCA	let-7f-1
327913	331	AACCGATTTCAAATGGTGCTAG	mir-29c
327914	332	GCCCTTTTAACATTGCACTG	mir-130a
327915	333	ACATGGTTAGATCAAGCACAA	mir-218-1
327916	334	TGGCATTCACCGCGTGCCTTAA	mir-124a-2
327917	335	TCAACATCAGTCTGATAAGCTA	mir-21
327918	336	CTAGTACATCATCTATACTGTA	mir-144
327919	337	GAAACCCAGCAGACAATGTAGCT	mir-221
327920	338	GAGACCCAGTAGCCAGATGTAGCT	mir-222
327921	339	CTTCCAGTCGGGGATGTTTACA	mir-30d
327922	340	TCAGTTTTGCATGGATTTGCACA	mir-19b-2
327923	341	GAAAGAGACCGGTTCACTGTGA	mir-128b
327924	342	GCAAGCCCAGACCGCAAAAAG	mir-129-2
327925	343	TAGCTGGTTGAAGGGGACCAA	mir-133b
327926	344	ACTATGCAACCTACTACCTCT	let-7d

327927	345	TGTAAACCATGATGTGCTGCTA	mir-15b
327928	346	AACCGATTTCAGATGGTGCTAG	mir-29a-1
327929	347	GAACAGATAGTCTAAACACTGGG	mir-199b
327930	348	ACTATACAACCTCCTACCTCA	let-7e
327931	349	AACCATACAACCTACTACCTCA	let-7c
327932	350	AGGCATAGGATGACAAAGGGAA	mir-204
327933	351	AAGGGATTCCTGGGAAAACTGGAC	mir-145
327934	352	GGTACAATCAACGGTCGATGGT	mir-213
327935	353	CTACCTGCACTATAAGCACTTTA	mir-20
327936	354	ACAGCTGGTTGAAGGGGACCAA	mir-133a-1
327937	355	GATTCACAACACCAGCT	mir-138-2
327938	356	AACAATACAACTTACTACCTCA	mir-98
327939	357	TCACAAGTTAGGGTCTCAGGGA	mir-125b-1
327940	358	GAACAGGTAGTCTGAACACTGGG	mir-199a-2
327941	359	AACCCACCGACAGCAATGAATGTT	mir-181b
327942	360	CCATCTTTACCAGACAGTGTT	mir-141
327943	361	TATCTGCACTAGATGCACCTTA	mir-18
327944	362	AAAGTGTCAGATACGGTGTGG	mir-220
327945	363	CTGTTCCTGCTGAACTGAGCCA	mir-24-2
327946	364	AGGCGAAGGATGACAAAGGGAA	mir-211
327947	365	TCAGTTATCACAGTACTGTA	mir-101-3
327948	366	GCTGAGTGTAGGATGTTTACA	mir-30b
327949	367	CACAAATTCGGATCTACAGGGTA	mir-10a
327950	368	TCAGTTTTGCATAGATTTGCACA	mir-19a
327951	369	CACAAACCATTATGTGCTGCTA	mir-15a-1
327952	370	CTACGCGTATTCTTAAGCAATA	mir-137
327953	371	AGAATTGCGTTTGGACAATCA	mir-219
327954	372	ACAAAGTTCTGTGATGCACTGA	mir-148b
327955	373	GCCCTTTCATCATTGCACTG	mir-130b
327956	374	CACAGTTGCCAGCTGAGATTA	mir-216
327957	375	CACAAGTTCGGATCTACGGGTT	mir-100-1
327958	376	CCGGCTGCAACACAAGACACGA	mir-187
327959	377	CAGCCGCTGTCACACGCACAG	mir-210
327960	378	GTCTGTCAATTCATAGGTCAT	mir-215
327961	379	GGGGTATTTGACAAACTGACA	mir-223
327962	380	GCTGAGAGTGTAGGATGTTTACA	mir-30c
327963	381	AACCTATCCTGAATTACTTGAA	mir-26b
327964	382	CCAAGTTCTGTCATGCACTGA	mir-152
327965	383	ATCACATAGGAATAAAAAGCCATA	mir-135-1
327966	384	ATCCAATCAGTTCCTGATGCAGTA	mir-217
327967	385	ACTGTACAAACTACTACCTCA	let-7g
327968	386	CAATGCAACAGCAATGCAC	mir-33b
327969	387	TGTGAGTTCTACCATTGCCAAA	mir-182
327970	388	ACAAAGTTCTGTAGTGCACTGA	mir-148a
327971	389	GGAAATCCCTGGCAATGTGAT	mir-23a
327972	390	ACTCACCGACAGGTTGAATGTT	mir-181c

Example 6: Uniform 2'-MOE phosphorothioate (PS) oligomeric compounds targeting novel miRNAs

In accordance with the present invention, a series of oligomeric compounds were designed and synthesized to target novel miRNAs. The compounds are shown in Table 4. "Pri-miRNA" indicates the particular pri-miRNA defined herein which contains the miRNA that the oligomeric compound was designed to target. The sequence of the compounds represent the full complement of the novel miRNA defined herein. All compounds in Table 4 are composed of 2'-

methoxyethoxy (2'-MOE) nucleotides throughout and the internucleoside (backbone) linkages are phosphorothioate (P=S) throughout. All cytidine residues are 5-methylcytidines. The compounds can be analyzed for their effect miRNA, pre-miRNA or pri-miRNA levels by quantitative real-time PCR as described, *supra*, or they can be used in other assays to investigate the role of miRNAs or downstream targets of miRNAs.

Table 4

Uniform 2'-MOE PS Compounds targeting novel pri-miRNAs

ISIS Number	SEQ ID NO	Sequence (5'-3')	Pri-miRNA
328089	391	ACTGTAGGAATATGTTTGATA	hypothetical miRNA-013
328090	392	ATTAAAAAGTCCTCTTGCCCA	hypothetical miRNA-023
328091	393	GCTGCCGTATATGTGATGTCA	hypothetical miRNA-030
328092	394	GGTAGGTGGAATACTATAACA	hypothetical miRNA-033
328093	395	TAAACATCACTGCAAGTCTTA	hypothetical miRNA-039
328094	396	TTGTAAGCAGTTTTGTTGACA	hypothetical miRNA-040
328095	397	TCACAGAGAAAACAACTGGTA	hypothetical miRNA-041
328096	398	CCTCTCAAAGATTTCCTGTCA	hypothetical miRNA-043
328097	399	TGTCAGATAAACAGAGTGGAA	hypothetical miRNA-044
328098	400	GAGAATCAATAGGGCATGCAA	hypothetical miRNA-055
328099	401	AAGAACATTAAGCATCTGACA	hypothetical miRNA-058
328100	402	AATCTCTGCAGGCAAATGTGA	hypothetical miRNA-070
328101	403	AAACCCCTATCACGATTAGCA	hypothetical miRNA-071
328102	404	GCCCCATTAATATTTTAACCA	hypothetical miRNA-075
328103	405	CCCAATATCAAACATATCA	hypothetical miRNA-079
328104	406	TATGATAGCTTCCCCATGTAA	hypothetical miRNA-083
328105	407	CCTCAATTATTGGAAATCACA	hypothetical miRNA-088
328106	408	ATTGATGCGCCATTTGGCCTA	hypothetical miRNA-090
328107	409	CTGTGACTTCTCTATCTGCCT	hypothetical miRNA-099
328108	410	AAACTTGTTAATTGACTGTCA	hypothetical miRNA-101
328109	411	AAAGAAGTATATGCATAGGAA	hypothetical miRNA-105
328110	412	GATAAAGCCAATAAACTGTCA	hypothetical miRNA-107
328111	413	TCCGAGTCGGAGGAGGAGGAA	hypothetical miRNA-111
328112	414	ATCATTACTGGATTGCTGTAA	hypothetical miRNA-120
328113	415	CAAAAATTATCAGCCAGTTTA	hypothetical miRNA-137
328114	416	AATCTCATTTTCATACTTGCA	hypothetical miRNA-138
328115	417	AGAAGGTGGGGAGCAGCGTCA	hypothetical miRNA-142
328116	418	CAAAATTGCAAGCAAATTGCA	hypothetical miRNA-143
328117	419	TCCACAAAGCTGAACATGTCT	hypothetical miRNA-144
328118	420	TATTATCAGCATCTGCTTGCA	hypothetical miRNA-153
328119	421	AATAACACACATCCACTTTAA	hypothetical miRNA-154
328120	422	AAGAAGGAAGGAGGGAAAGCA	hypothetical miRNA-156
328121	423	ATGACTACAAGTTTATGGCCA	hypothetical miRNA-161
328122	424	CAAAACATAAAAATCCTTGCA	hypothetical miRNA-164
328123	425	TTACAGGTGCTGCAACTGGAA	hypothetical miRNA-166
328124	426	AGCAGGTGAAGGCACCTGGCT	hypothetical miRNA-168
328125	427	TATGAAATGCCAGAGCTGCCA	hypothetical miRNA-169
328126	428	CCAAGTGTTAGAGCAAGATCA	hypothetical miRNA-170
328127	429	AACGATAAAACATACTTGTCA	hypothetical miRNA-171
328128	430	AGTAACTTCTTGCAGTTGGA	hypothetical miRNA-172
328129	431	AGCCTCCTTCTTCTCGTACTA	hypothetical miRNA-173
328130	432	ACCTCAGGTGGTTGAAGGAGA	hypothetical miRNA-175
328131	433	ATATGTCATATCAAACTCCTA	hypothetical miRNA-176
328132	434	GTGAGAGTAGCATGTTTGTCT	hypothetical miRNA-177
328133	435	TGAAGGTTCGGAGATAGGCTA	hypothetical miRNA-178

328134	436	AATTGGACAAAGTGCCTTTCA	hypothetical miRNA-179
328135	437	ACCGAACAAAGTCTGACAGGA	hypothetical miRNA-180
328136	438	AACTACTTCCAGAGCAGGTGA	hypothetical miRNA-181
328137	439	GTAAGCGCAGCTCCACAGGCT	hypothetical miRNA-183
328138	440	GAGCTGCTCAGCTGGCCATCA	hypothetical miRNA-185
328139	441	TACTTTTCATTCCCCTCACCA	hypothetical miRNA-188

Example 7: Chimeric phosphorothioate compounds having 2'-MOE wings and a deoxy gap targeting pri-miRNAs

In accordance with the present invention, a series of oligomeric compounds were designed and synthesized to target different regions of pri-miRNA structures. The compounds are shown in Table 5. "Pri-miRNA" indicates the particular pri-miRNA which contains the miRNA that the oligomeric compound was designed to target. All compounds in Table 5 are chimeric oligonucleotides ("gapmers") 20 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide "wings." The wings are composed of 2'-methoxyethoxy (2'-MOE) nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines. The compounds can be analyzed for their effect on miRNA, pre-miRNA or pri-miRNA levels by quantitative real-time PCR as described, *supra*, or they can be used in other assays to investigate the role of miRNAs or miRNA downstream targets.

Table 5

Chimeric phosphorothioate oligomeric compounds having 2'-MOE wings and a deoxy gap targeting pri-miRNAs

ISIS Number	SEQ ID NO	Sequence	pri-miRNA
328333	442	AGAACAGCATGACGTAACCT	mir-140
328334	443	GCCCATCTGTGGCTTCACAG	mir-30a
328335	444	GAAGTCCGAGGCAGTAGGCA	mir-30a
328336	445	CTTCCTTACTATTGCTCACA	mir-34
328337	446	GCTAGATACAAAGATGGAAA	mir-29b-1
328338	447	CTAGACAATCACTATTTAAA	mir-29b-2
328339	448	GCAGCGCAGCTGGTCTCCCC	mir-29b-2
328340	449	TAATATATATTTCACTACGC	mir-16-3
328341	450	TGCTGTATCCCTGTCACACT	mir-16-3
328342	451	CAATTGCGCTACAGAACTGT	mir-203
328343	452	TCGATTTAGTTATCTAAAAA	mir-7-1
328344	453	CTGTAGAGGCATGGCCTGTG	mir-7-1
328345	454	TGACTATACGGATACCACAC	mir-10b
328346	455	GGAACAAGGCCAATTATTGC	mir-128a
328347	456	AGAAATGTAAACCTCTCAGA	mir-128a
328348	457	AGCTGTGAGGGAGAGAGAGA	mir-153-1
328349	458	CTGGAGTGAGAATACTAGCT	mir-153-1
328350	459	ACTGGGCTCATATTACTAGC	mir-153-2
328351	460	TTGGATTAAATAACAACCTA	hypothetical miRNA-013

328352	461	CCCGGAGACAGGGCAAGACA	hypothetical miRNA-013
328353	462	AAAGCGGAAACCAATCACTG	mir-27b
328354	463	GTCCCCATCTCACCTTCTCT	mir-27b
328355	464	TCAGAGCGGAGAGACACAAG	mir-96
328356	465	TAGATGCACATATCACTACC	mir-17as/mir-91
328357	466	CTTGGCTTCCCGAGGCAGCT	mir-17as/mir-91
328358	467	AGTTTGAAGTGTCACAGCGC	mir-123/mir-126as
328359	468	GCGTTTTCGATGCGGTGCCG	mir-123/mir-126as
328360	469	GAGACGCGGGGGCGGGGCGC	mir-132
328361	470	TACCTCCAGTTCCCACAGTA	mir-132
328362	471	TGTGTTTTCTGACTCAGTCA	mir-108-1
328363	472	AGAGCACCTGAGAGCAGCGC	mir-23b
328364	473	TCTTAAGTCACAAATCAGCA	mir-23b
328365	474	TCTCCACAGCGGGCAATGTC	let-7i
328366	475	GGCGCGCTGTCCGGGCGGGG	mir-212
328367	476	ACTGAGGGCGGCCCGGGCAG	mir-212
328368	477	GTCCTCTTGCCCAAGCAACA	hypothetical miRNA-023
328369	478	GAAGACCAATACACTCATAC	mir-131-2
328370	479	CCGAGGGGCAACATCACTGC	let-7b
328371	480	TCCATAGCTTAGCAGGTCCA	mir-1d
328372	481	TTTGATAGTTTAGACACAAA	mir-122a
328373	482	GGGAAGGATTGCCTAGCAGT	mir-122a
328374	483	AGCTTTAGCTGGGTCAGGAC	mir-22
328375	484	TACCATACAGAAACACAGCA	mir-92-1
328376	485	TCACAATCCCCACCAAACTC	mir-92-1
328377	486	TCACTCCTAAAGGTTCAAGT	hypothetical miRNA-030
328378	487	CACCCTCCAGTGCTGTTAGT	mir-142
328379	488	CTGACTGAGACTGTTCACAG	mir-183
328380	489	CCTTTAGGGGTTGCCACACC	hypothetical miRNA-033
328381	490	ACAGGTGAGCGGATGTTCTG	mir-214
328382	491	CAGACTCCCAACTGACCAGA	mir-143
328383	492	AGAGGGGAGACGAGAGCACT	mir-192-1
328384	493	TCACGTGGAGAGGAGTTAAA	hypothetical miRNA-039
328385	494	AGTGCTAATACTTCTTTCAT	hypothetical miRNA-040
328386	495	ACCTGTGTAACAGCCGTGTA	hypothetical miRNA-041
328387	496	TTATCGGAACTTCACAGAGA	hypothetical miRNA-041
328388	497	TCCCATAGCAGGGCAGAGCC	let-7a-3
328389	498	GGCACTTCATTGCTGCTGCC	hypothetical miRNA-043
328390	499	GGAGCCTTGCGCTCAGCATT	hypothetical miRNA-043
328391	500	ATGGTAATTTCATTTCAGGC	hypothetical miRNA-044
328392	501	GATTGCACATCCACACTGTC	hypothetical miRNA-044
328393	502	GCTGGCCTGATAGCCCTTCT	mir-181a
328394	503	GTTTTTTCAAATCCCAAACT	mir-181a
328395	504	CCCAGTGGTGGGTGTGACCC	let-7a-1
328396	505	CTGGTTGGGTATGAGACAGA	mir-205
328397	506	TTGATCCATATGCAACAAGG	mir-103-1
328398	507	GCCATTGGGACCTGCACAGC	mir-26a
328399	508	ATGGGTACCACCAGAACATG	mir-33a
328400	509	AGTTCAAAACTCAATCCCAA	mir-196-2
328401	510	GCCCTCGACGAAAACCGACT	mir-196-2
328402	511	TTGAACTCCATGCCACAAGG	mir-107
328403	512	AGGCCTATTCCTGTAGCAAA	mir-106
328404	513	GTAGATCTCAAAAAGCTACC	mir-106
328405	514	CTGAACAGGGTAAAATCACT	let-7f-1
328406	515	AGCAAGTCTACTCCTCAGGG	let-7f-1
328407	516	AATGGAGCCAAGGTGCTGCC	hypothetical miRNA-055
328408	517	TAGACAAAAACAGACTCTGA	mir-29c
328409	518	GCTAGTGACAGGTGCAGACA	mir-130a
328410	519	GGGCCTATCCAAAGTGACAG	hypothetical miRNA-058

328411	520	TACCTCTGCAGTATTCTACA	hypothetical miRNA-058
328412	521	TTTACTCATACCTCGCAACC	mir-218-1
328413	522	AATTGTATGACATTAAATCA	mir-124a-2
328414	523	CTTCAAGTGCAGCCGTAGGC	mir-124a-2
328415	524	TGCCATGAGATTCAACAGTC	mir-21
328416	525	ACATTGCTATCATAAGAGCT	mir-16-1
328417	526	TAATTTTAGAATCTTAACGC	mir-16-1
328418	527	AGTGTCTCATCGCAAACTTA	mir-144
328419	528	TGTTGCCTAACGAACACAGA	mir-221
328420	529	GCTGATTACGAAAGACAGGA	mir-222
328421	530	GCTTAGCTGTGTCTTACAGC	mir-30d
328422	531	GAGGATGTCTGTGAATAGCC	mir-30d
328423	532	CCACATATACATATATACGC	mir-19b-2
328424	533	AGGAAGCACACATTATCACA	mir-19b-2
328425	534	GACCTGCTACTCACTCTCGT	mir-128b
328426	535	GGTTGGCCGCAGACTCGTAC	hypothetical miRNA-069
328427	536	GATGTCACTGAGGAAATCAC	hypothetical miRNA-070
328428	537	TCAGTTGGAGGCAAAAACCC	hypothetical miRNA-071
328429	538	GGTAGTGCAGCGCAGCTGGT	mir-29b-3
328430	539	CCGGCTATTGAGTTATGTAC	mir-129-2
328431	540	ACCTCTCAGGAAGACGGACT	mir-133b
328432	541	GAGCATGCAACACTCTGTGC	hypothetical miRNA-075
328433	542	CCTCCTTGTGGGCAAAATCC	let-7d
328434	543	CGCATCTTGACTGTAGCATG	mir-15b
328435	544	TCTAAGGGGTCACAGAAGGT	mir-29a-1
328436	545	GAAAATTATATTGACTCTGA	mir-29a-1

Example 8: Chimeric phosphorothioate compounds having 2'-MOE wings and a deoxy gap targeting pri-miRNAs

In accordance with the present invention, a second series of oligomeric compounds were designed and synthesized to target different regions of pri-miRNA structures. The compounds are shown in Table 6. "Pri-miRNA" indicates the particular pri-miRNA which contains the miRNA that the oligomeric compound was designed to target. All compounds in Table 6 are chimeric oligonucleotides ("gapmers") 20 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide "wings." The wings are composed of 2'-methoxyethoxy (2'-MOE) nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines. The compounds can be analyzed for their effect on miRNA, pre-miRNA or pri-miRNA levels by quantitative real-time PCR as described, *supra*, or they can be used in other assays to investigate the role of miRNAs or miRNA downstream targets.

Table 6

Chimeric phosphorothioate oligomeric compounds having 2'-MOE wings and a deoxy gap targeting pri-miRNAs

ISIS Number	SEQ ID NO	Sequence	pri-miRNA
328637	546	GGTTCCTAATTAAACAACCC	hypothetical miRNA-079
328638	547	CCGAGGGTCTAACCCAGCCC	mir-199b
328639	548	GACTACTGTTGAGAGGAACA	mir-129-1
328640	549	TCTCCTTGGGTGTCCTCCTC	let-7e
328641	550	TGCTGACTGCTCGCCCTTGC	hypothetical miRNA-083
328642	551	ACTCCCAGGGTGTAACTCTA	let-7c
328643	552	CATGAAGAAAGACTGTAGCC	mir-204
328644	553	GACAAGGTGGGAGCGAGTGG	mir-145
328645	554	TGCTCAGCCAGCCCCATTCT	mir-124a-1
328646	555	GCTTTTAGAACCACTGCCTC	hypothetical miRNA-088
328647	556	GGAGTAGATGATGGTTAGCC	mir-213
328648	557	ACTGATTCAAGAGCTTTGTA	hypothetical miRNA-090
328649	558	GTAGATAACTAAACACTACC	mir-20
328650	559	AATCCATTGAAGAGGCGATT	mir-133a-1
328651	560	GGTAAGAGGATGCGCTGCTC	mir-138-2
328652	561	GGCCTAATATCCCTACCCCA	mir-98
328653	562	GTGTTCAGAAACCCAGGCCC	mir-196-1
328654	563	TCCAGGATGCAAAAGCACGA	mir-125b-1
328655	564	TACAACGGCATTGTCCTGAA	mir-199a-2
328656	565	TTTCAGGCTCACCTCCCCAG	hypothetical miRNA-099
328657	566	AAAAATAATCTCTGCACAGG	mir-181b
328658	567	AGAATGAGTTGACATACCAA	hypothetical miRNA-101
328659	568	GCTTCACAATTAGACCATCC	mir-141
328660	569	AGACTCCACACCACTCATAC	mir-131-1
328661	570	ATCCATTGGACAGTCGATTT	mir-133a-2
328662	571	GGCGGGCGGCTCTGAGGCGG	hypothetical miRNA-105
328663	572	CTCTTTAGGCCAGATCCTCA	hypothetical miRNA-106
328664	573	TAATGGTATGTGTGGTGATA	hypothetical miRNA-107
328665	574	ATTACTAAGTTGTTAGCTGT	mir-1b
328666	575	GATGCTAATCTACTTCACTA	mir-18
328667	576	TCAGCATGGTGCCCTCGCCC	mir-220
328668	577	TCCGCGGGGGCGGGGAGGCT	hypothetical miRNA-111
328669	578	AGACCACAGCCACTCTAATC	mir-7-3
328670	579	TCCGTTTCCATCGTTCCACC	mir-218-2
328671	580	GCCAGTGTACACAAACCAAC	mir-24-2
328672	581	AAGGCTTTTTGCTCAAGGGC	mir-24-1
328673	582	TTGACCTGAATGCTACAAGG	mir-103-2
328674	583	TGCCCTGCTCAGAGCCCTAG	mir-211
328675	584	TCAATGTGATGGCACCACCA	mir-101-3
328676	585	ACCTCCCAGCCAATCCATGT	mir-30b
328677	586	TCCTGGATGATATCTACCTC	hypothetical miRNA-120
328678	587	TCTCCCTTGATGTAATTCTA	let-7a-4
328679	588	AGAGCGGAGTGTTTATGTCA	mir-10a
328680	589	TCATTCATTTGAAGGAAATA	mir-19a
328681	590	TCCAAGATGGGGTATGACCC	let-7f-2
328682	591	TTTTTAAACACACATTCGCG	mir-15a-1
328683	592	AGATGTGTTTCCATTCCACT	mir-108-2
328684	593	CCCCCTGCCGCTGGTACTCT	mir-137
328685	594	CGGCCGGAGCCATAGACTCG	mir-219
328686	595	CTTTCAGAGAGCCACAGCCT	mir-148b
328687	596	GCTTCCCAGCGGCCTATAGT	mir-130b
328688	597	CAGCAGAATATCACACAGCT	mir-19b-1
328689	598	TACAATTTGGGAGTCCTGAA	mir-199b
328690	599	GCCTCCTTCATATATTCTCA	mir-204
328691	600	CCCCATCTTAGCATCTAAGG	mir-145
328692	601	TTGTATGGACATTTAAATCA	mir-124a-1
328693	602	TTTGATTTTAATTCCAAACT	mir-213

328694	603	CAAACGGTAAGATTTGCAGA	hypothetical miRNA-090
328695	604	GGATTTAAACGGTAAACATC	mir-125b-1
328696	605	CTCTAGCTCCCTCACCAGTG	hypothetical miRNA-099
328697	606	GCTTGTCCACACAGTTCAAC	mir-181b
328698	607	GCATTGTATGTTCATATGGG	mir-1b
328699	608	TGTCGTAGTACATCAGAACA	mir-7-3
328700	609	AGCCAGTGTGTAAAATGAGA	mir-24-1
328701	610	TTCAGATATACAGCATCGGT	mir-101-3
328702	611	TGACCACAAAATTCCTTACA	mir-10a
328703	612	ACAACTACATTCTTCTTGTA	mir-19a
328704	613	TGCACCTTTTCAAAATCCAC	mir-15a-1
328705	614	AACGTAATCCGTATTATCCA	mir-137

Example 9: Chimeric phosphorothioate compounds having 2'-MOE wings and a deoxy gap targeting pri-miRNAs

In accordance with the present invention, a third series of oligomeric compounds were designed and synthesized to target different pri-miRNA structures. The compounds are shown in Table 7. "Pri-miRNA" indicates the particular pri-miRNA which contains the miRNA that the oligomeric compound was designed to target. All compounds in Table 7 are chimeric oligonucleotides ("gapmers") 20 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide "wings." The wings are composed of 2'-methoxyethoxy (2'-MOE) nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines. The compounds can be analyzed for their effect on miRNA, pre-miRNA or pri-miRNA levels by quantitative real-time PCR as described, supra, or they can be used in other assays to investigate the role of miRNAs or miRNA downstream targets.

Table 7

Chimeric phosphorothioate oligomeric compounds having 2'-MOE wings and a deoxy gap targeting pri-miRNAs

ISIS Number	SEQ ID NO	Sequence	pri-miRNA
328706	615	CGTGAGGGCTAGGAAATTGC	mir-216
328707	616	GCAACAGGCCTCAATATCTT	mir-100-1
328708	617	ACGAGGGGTCAGAGCAGCGC	mir-187
328709	618	GGCAGACGAAAGGCTGACAG	hypothetical miRNA-137
328710	619	CTGCACCATGTTCGGCTCCC	hypothetical miRNA-138
328711	620	GGGGCCCTCAGGGCTGGGGC	mir-124a-3
328712	621	CCGGTCCACTCTGTATCCAG	mir-7-2
328713	622	GCTGGGAAAGAGAGGGCAGA	hypothetical miRNA-142
328714	623	TCAGATTGCCAACATTGTGA	hypothetical miRNA-143
328715	624	CTGGGGAGGGGGTTAGCGTC	hypothetical miRNA-144
328716	625	TGGGTCTGGGGCAGCGCAGT	mir-210
328717	626	TTGAAGTAGCACAGTCATAC	mir-215
328718	627	TCTACCACATGGAGTGTCCA	mir-124a-3
328719	628	AGTGCCGCTGCCGCGCCGTG	mir-7-2

328720	629	ACACATTGAGAGCCTCCTGA	hypothetical miRNA-142
328721	630	GTCGCTCAGTGCTCTCTAGG	hypothetical miRNA-143
328722	631	AGGCTCCTCTGATGGAAGGT	hypothetical miRNA-144
328723	632	GCTGTGACTTCTGATATTAT	hypothetical miRNA-153
328724	633	GACATCATGTGATTTGCTCA	hypothetical miRNA-154
328725	634	CACCCCAAGGCTGCAGGGCA	mir-26b
328726	635	TGTCAAGCCTGGTACCACCA	hypothetical miRNA-156
328727	636	CTGCTCCAGAGCCCGAGTCG	mir-152
328728	637	ACCCTCCGCTGGCTGTCCCC	mir-135-1
328729	638	TAGAGTGAATTTATCTTGGT	mir-135-2
328730	639	TGGTGACTGATTCTTATCCA	mir-217
328731	640	CAATATGATTGGATAGAGGA	hypothetical miRNA-161
328732	641	TTTAAACACACATTCGCGCC	mir-15a-2
328733	642	ACCGGGTGGTATCATAGACC	let-7g
328734	643	TGCATACCTGTTCAGTTGGA	hypothetical miRNA-164
328735	644	GCCCGCCTCTCTCGGCCCCC	mir-33b
328736	645	TCGCCCCCTCCCAGGCCTCT	hypothetical miRNA-166
328737	646	ACAACTGTAGAGTATGGTCA	mir-16-2
328738	647	GCTGACCATCAGTACTTTCC	hypothetical miRNA-168
328739	648	TTATAGAACAGCCTCCAGTG	hypothetical miRNA-169
328740	649	TTCAGGCACTAGCAGTGGGT	hypothetical miRNA-170
328741	650	AGTACTGCGAGGTTAACCGC	hypothetical miRNA-171
328742	651	GGACCTTTAAGATGCAAAGT	hypothetical miRNA-172
328743	652	TTCATATTATCCACCCAGGT	hypothetical miRNA-173
328744	653	CGGATCCTGTTACCTCACCA	mir-182
328745	654	TGGTGCCTGCCACATCTTTG	hypothetical miRNA-175
328746	655	TGGGAGGCTGAATCAAGGAC	hypothetical miRNA-176
328747	656	TGACAACCAGGAAGCTTGTG	hypothetical miRNA-177
328748	657	GCCAGGCAGCGAGCTTTTGA	hypothetical miRNA-178
328749	658	CAGCCTGCCACCGCCGCTTT	hypothetical miRNA-179
328750	659	CTGCCCCCGTGGACCGAACA	hypothetical miRNA-180
328751	660	TCGTGCACCTGAGGAGTCTG	hypothetical miRNA-181
328752	661	CAAACGTGCTGTCTTCCTCC	mir-148a
328753	662	AAGGACTCAGCAGTGTTTCA	hypothetical miRNA-183
328754	663	TCCTCGGTGGCAGAGCTCAG	mir-23a
328755	664	AGACAATGAGTACACAGTTC	hypothetical miRNA-185
328756	665	CTGCAAGCACTGGTTCCCAT	hypothetical miRNA-186
328757	666	TTGCCTGAGCTGCCCAAACT	mir-181c
328758	667	TCCATCACACTGTCCTATGA	hypothetical miRNA-188
328759	668	GAGGGATTGTATGAACATCT	mir-216
328760	669	GCTTGTGCGGACTAATACCA	mir-100-1
328761	670	GCAGGCTAAAAGAAATAAGC	hypothetical miRNA-138
328762	671	ATTGTATAGACATTAAATCA	mir-124a-3
328763	672	GTTGAGCGCAGTAAGACAAC	mir-7-2
328764	673	AGATGTTTCTGGCCTGCGAG	hypothetical miRNA-142
328765	674	GACAAACTCAGCTATATTGT	mir-215
328766	675	ACGGCTCTGTGGCACTCATA	mir-131-3
328767	676	GCTTTCTTACTTTCCACAGC	mir-30c
328768	677	TACCTTTAGAATAGACAGCA	mir-101-1
328769	678	AGGCTGGACAGCACACAACC	mir-26b
328770	679	AGCAGGAGCCTTATCTCTCC	hypothetical miRNA-156
328771	680	ATGAGTGAGCAGTAGAATCA	mir-135-1
328772	681	TGAGACTTTATTACTATCAC	mir-135-2
328773	682	TACTTTACTCCAAGGTTTTA	mir-15a-2
328774	683	GCACCCGCCTCACACACGTG	mir-33b
328775	684	TTCCCGACCTGCCTTTACCT	hypothetical miRNA-166
328776	685	TCCTGTAATTATAGGCTAGC	hypothetical miRNA-169
328777	686	GGATCATATCAATAATACCA	hypothetical miRNA-172
328778	687	TGCTGAGACACACAATATGT	hypothetical miRNA-176

328779	688	TGTTTGTCTCCAAGAAACGT	hypothetical miRNA-177
328780	689	TGTCATGGACAGGATGAATA	hypothetical miRNA-179
328781	690	TCTATCATACTCAGAGTCGG	mir-148a
328782	691	TTGTGACAGGAAGCAAATCC	mir-23a
328783	692	CATCAGAGTCACCAACCCCA	hypothetical miRNA-185
328784	693	CAAGAGATGTCTCGTTTTGC	hypothetical miRNA-186

Example 10: Chimeric phosphorothioate compounds having 2'-MOE wings and a deoxy gap targeted to the stem loop of pri-miRNA structures

In accordance with the present invention, a fourth series of oligomeric compounds were designed to target the stem loop of different pri-miRNA structures. In some cases, these oligomeric compounds contain mismatches, and thus hybridize with partial complementarity to the stemloop structure of the pri-miRNA targeted. The compounds are shown in Table 8. "Pri-miRNA" indicates the particular pri-miRNA that the oligomeric compound was designed to target. All compounds in Table 8 are chimeric oligonucleotides ("gapmers"), composed of a central "gap" region consisting of 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by "wings." The wings are composed of 2'-methoxyethoxy (2'-MOE) nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines. The compounds can be analyzed for their effect on miRNA, pre-miRNA or pri-miRNA levels by quantitative real-time PCR as described, *supra*, or they can be used in other assays to investigate the role of miRNAs or downstream nucleic acid targets.

Table 8

Chimeric phosphorothioate compounds having 2'-MOE wings and a deoxy gap targeted to the stem loop of pri-miRNA structures

Compound Number	SEQ ID NO.	Sequence	Pri-miRNA
RG1	694	GTGGTAGAACAGCATGACGTC	mir-140
RG2	695	AGCTGTGAAGCCACGATGGGC	mir-30a
RG3	696	AGATACAAAGATGGAAAAATC	mir-29b-1
RG4	697	CTTCCTTACTATTGCTCACAA	mir-34
RG5	698	TGTTTAATATATATTTCACTC	mir-16-3
RG6	699	TGTCAAGACATCGCGTTAACA	mir-203
RG7	700	TGTCGATTTAGTTATCCAACA	mir-7-1
RG8	701	GTGACTATACGGATACCACAC	mir-10b
RG9	702	ACCTCTCCAAATGTAAAGA	mir-128a
RG10	703	CAAAGCGGAAACCAATCACTG	mir-27b
RG11	704	CTGCAGTACATGCACATATCA	mir-91
RG12	705	AACAATGACACCCTTGACCT	mir-132
RG13	706	TTTTAATCTTAAGTCACAAA	mir-23b
RG14	707	ATCTCCACAGCGGGCAATGTC	let-7i
RG15	708	TATGAAGACCAATACACTCCA	mir-131-2
RG16	709	GGGGCAACATCACTGCCC	let-7b

RG17	710	CCATGTTAGCAGGTCCATATG	mir-1d
RG18	711	GTTTGATAGTTTAGACACAAA	mir-122a
RG19	712	TGGGTCAGGACTAAAGCTTC	mir-22
RG20	713	AATACCATACAGAAACACAGC	mir-92-1
RG21	714	TTCGTGATGATTGTCGTGCC	mir-142
RG22	715	ACTGCGAGACTGTTCACAGTT	mir-183
RG23	716	TACAGGTGAGCGGATGTTCTG	mir-214
RG24	717	TCTCAGCTCCCAACTGACCAG	mir-143
RG25	718	ACCGCAGATATTACAGCCACT	let-7a-3
RG26	719	CCTGATAGCCCTTCTTAAGGA	mir-181a
RG27	720	CTTGATCCATATGCAACAAGG	mir-103-1
RG28	721	GCCATTGGGACCTGCACACC	mir-26a
RG29	722	GCATGGGTACCACCCCATGC	mir-33a
RG30	723	CGAGTTCAAAACTCAATCCCA	mir-196-2
RG31	724	CTTGAACTCCATGCCACAAGG	mir-107
RG32	725	GTAGATCTCAAAAAGCTAGC	mir-106
RG33	726	GAACAGGGTAAAATCACTAC	let-7f-1
RG34	727	AGACAAAAACAGACTCTGAA	mir-29c
RG35	728	GCTAGTGACAGGTCCAGACAG	mir-130a
RG36	729	TTTACTCATACCTCGCAACCA	mir-218-1
RG37	730	TTAATTGTATGACATTAAATCA	mir-124a-2
RG38	731	TGCCATGAGATTCAACAGTCA	mir-21
RG39	732	GATAATATTTAGAATCTTAAC	mir-16-1
RG40	733	TAGTGTCTCATCGCAAACTTA	mir-144
RG41	734	CTGTTGCCTAACGAACACAGA	mir-221
RG42	735	TGCTGATTACGAAAGACAGGAT	mir-222
RG43	736	GCTTAGCTGTGTCTTACAGCT	mir-30d

Example 11: Effects of oligomeric compounds targeting miRNAs on Apoptosis in Caspase Assay

Programmed cell death or apoptosis involves the activation of proteases, a family of intracellular proteases, through a cascade which leads to the cleavage of a select set of proteins. The caspase family contains at least 14 caspases, with differing substrate preferences. The caspase activity assay uses a DEVD peptide to detect activated caspases in cell culture samples. The peptide is labeled with a fluorescent molecule, 7-amino-4-trifluoromethyl coumarin (AFC). Activated caspases cleave the DEVD peptide resulting in a fluorescence shift of the AFC. Increased fluorescence is indicative of increased caspase activity and consequently increased cell death. The chemotherapeutic drugs taxol, cisplatin, etoposide, gemcitabine, camptothecin, aphidicolin and 5-fluorouracil all have been shown to induce apoptosis in a caspase-dependent manner.

The effect of several oligomeric compounds of the present invention was examined in cells expressing miRNA targets. The cells expressing the targets used in these experiments were T47D, a breast carcinoma cell line. Other cell lines can also be employed in this assay and these include normal human mammary epithelial cells (HMECs) as well as two breast carcinoma cell

lines, MCF7 and T47D. All of the cell lines were obtained from the American Type Culture Collection (Manassas, VA). The latter two cell lines express similar genes but MCF7 cells express the tumor suppressor p53, while T47D cells are deficient in p53. MCF-7 cells are routinely cultured in DMEM low glucose (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Gaithersburg, MD). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. T47D cells were cultured in Gibco DMEM High glucose media supplemented with 10% Fetal Bovine Serum (FBS).

Cells were plated at 10,000 cells per well for HMEC cells or 20,000 cells per well for MCF7 and T47D cells, and allowed to attach to wells overnight. Plates used were 96 well Costar plate 1603 (black sides, transparent bottom). DMEM high glucose medium, with and without phenol red, were obtained from Invitrogen (San Diego, CA). MEGM medium, with and without phenol red, were obtained from Biowhittaker (Walkersville, MD). The caspase-3 activity assay kit was obtained from Calbiochem (Cat. #HTS02) (EMD Biosciences, San Diego, CA).

Before adding to cells, the oligomeric compound cocktail was mixed thoroughly and incubated for 0.5 hrs. The oligomeric compound or the LIPOFECTIN™-only vehicle control was added (generally from a 3 μM stock of oligonucleotide) to a final concentration of 200nM with 6μg/ml LIPOFECTIN™. The medium was removed from the plates and the plates were tapped on sterile gauze. Each well was washed in 150 μl of PBS (150μL HBSS for HMEC cells). The wash buffer in each well was replaced with 100 μL of the oligomeric compound/OPTI-MEM™/LIPOFECTIN™ cocktail (this was T=0 for oligomeric compound treatment). The plates were incubated for 4 hours at 37° C, after which the medium was dumped and the plate was tapped on sterile gauze. 100 μl of full growth medium without phenol red was added to each well. After 48 hours, 50μl of oncogene buffer (provided with Calbiochem kit) with 10μM DTT was added to each well. 20μl of oncogene substrate (DEVD-AFC) was added to each well. The plates were read at 400 ± 25nm excitation and 508 ± 20nm emission at t=0 and t=3 time points. The t=0 x (0.8) time point was subtracted from the t=3 time point, and the data are shown as percent of LIPOFECTIN™-only (untreated control) treated cells.

Four experiments were performed and the results are shown in Tables 9-12. The concentration of oligomeric compound used was 200nM. All compounds in Tables 9-12 are chimeric oligomeric compounds ("gapmers") 20 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide "wings." The wings are composed of 2'-methoxyethoxy (2'-MOE)

nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the compound. All cytidine residues are 5-methylcytidines. As a control a 20-mer oligonucleotide random-mer, ISIS-29848 (NNNNNNNNNNNNNNNNNNNN; where N is A,T,C or G; herein incorporated as SEQ ID NO: 737) was used. In addition, two positive controls targeting expressed genes known to induce apoptosis when inhbited were included. These were ISIS-148715 (TTGTCCCAGTCCCAGGCCTC; herein incorporated as SEQ ID NO: 738) which targets human Jagged2 and ISIS-226844 (GCCCTCCATGCTGGCACAGG; herein incorporated as SEQ ID NO: 739) which targets human Notch1. Both positive controls have the same chemistry and gap structure as the compounds being tested. An increase in fluorescence indicates that the compound, by inhibiting its target, induces apoptosis as compared to untreated controls (UTC).

Table 9

Effects of oligomeric compounds targeting miRNAs on Apoptosis in Caspase Assay

ISIS Number	SEQ ID NO.	Pri-miRNA	Fold Increase over UTC
UTC Untreated control	N/A	N/A	1.0
ISIS-29848 n-mer	737	N/A	3.5
ISIS-148715 Positive control	738	Jagged2	1.5
ISIS-226844 Positive control	739	Notch1	3.6
328371	480	mir-1d	1.2
328400	509	mir-196-2	1.3
328420	529	mir-222	1.0
328692	601	mir-124a-1	1.2
328381	490	mir-214	1.1
328691	600	mir-145	0.9
328391	500	hypothetical miRNA-044	0.8
328415	524	mir-21	1.1
328433	542	let-7d	1.0
328643	552	mir-204	0.9
328377	486	hypothetical miRNA-030	0.7
328405	514	let-7f-1	1.0
328372	481	mir-122a	1.0
328403	512	mir-106	1.0
328424	533	mir-19b-2	0.9
328648	557	hypothetical miRNA-090	1.1
328397	506	mir-103-1	1.2
328656	565	hypothetical miRNA-099	1.1
328392	501	hypothetical miRNA-044	1.0
328421	530	mir-30d	1.2
328417	526	mir-16-1	1.0
328647	556	mir-213	0.9
328378	487	mir-142	1.0
328416	525	mir-16-1	0.9

Table 10

Effects of oligomeric compounds targeting miRNAs on Apoptosis in Caspase Assay

ISIS Number	SEQ ID NO.	Pri-miRNA	Fold Increase over UTC
UTC Untreated control	N/A	N/A	0.9
ISIS-29848 n-mer	737	N/A	3.0
ISIS-148715 Positive control	738	Jagged2	1.0
ISIS-226844 Positive control	739	Notch1	3.1
328375	484	mir-92-1	0.9
328382	491	mir-143	0.9
328383	492	mir-192-1	1.2
328385	494	hypothetical miRNA-040	0.9
328395	504	let-7a-1	1.0
328398	507	mir-26a	0.9
328399	508	mir-33a	1.0
328402	511	mir-107	1.2
328408	517	mir-29c	0.9
328409	518	mir-130a	0.7
328422	531	mir-30d	1.0
328423	532	mir-19b-2	0.6
328425	534	mir-128b	0.8
328431	540	mir-133b	0.9
328436	545	mir-29a-1	0.9
328646	555	hypothetical miRNA-088	1.1
328649	558	mir-20	1.0
328651	560	mir-138-2	0.9
328652	561	mir-98	1.2
328657	566	mir-181b	0.8
328672	581	mir-24-1	0.9
328694	603	hypothetical miRNA-090	0.8
328696	605	hypothetical miRNA-099	1.5
328700	609	mir-24-1	0.8

Table 11

Effects of oligomeric compounds targeting miRNAs on Apoptosis in Caspase Assay

ISIS Number	SEQ ID NO.	Pri-miRNA	Fold Increase over UTC
UTC Untreated control	N/A	N/A	0.9
ISIS-29848 n-mer	737	N/A	3.2
ISIS-148715 Positive control	738	Jagged2	1.1
ISIS-226844 Positive control	739	Notch1	3.1
328374	483	mir-22	1.1
328376	485	mir-92-1	0.7
328384	493	hypothetical miRNA-039	1.0
328386	495	hypothetical miRNA-041	0.7
328390	499	hypothetical miRNA-043	0.9
328393	502	mir-181a	1.5
328404	513	mir-106	0.9

328406	515	let-7f-1	1.0
328407	516	hypothetical miRNA-055	1.2
328410	519	hypothetical miRNA-058	1.5
328411	520	hypothetical miRNA-058	0.8
328413	522	mir-124a-2	0.8
328426	535	hypothetical miRNA-069	1.3
328427	536	hypothetical miRNA-070	0.8
328435	544	mir-29a-1	1.3
328637	546	hypothetical miRNA-079	1.0
328638	547	mir-199b	0.8
328639	548	mir-129-1	0.8
328645	554	mir-124a-1	2.2
328653	562	mir-196-1	1.1
328654	563	mir-125b-1	1.0
328655	564	mir-199a-2	0.7
328689	598	mir-199b	0.8
328695	604	mir-125b-1	0.8

Table 12

Effects of oligomeric compounds targeting miRNAs on Apoptosis in Caspase Assay

ISIS Number	SEQ ID NO.	Pri-miRNA	Fold Increase over UTC
UTC Untreated control	N/A	N/A	1.0
ISIS-29848 n-mer	737	N/A	3.5
ISIS-148715 Positive control	738	Jagged2	1.3
ISIS-226844 Positive control	739	Notch1	3.5
328373	482	mir-122a	0.9
328379	488	mir-183	1.1
328387	496	hypothetical miRNA-041	1.4
328388	497	let-7a-3	0.9
328389	498	hypothetical miRNA-043	1.1
328394	503	mir-181a	0.8
328396	505	mir-205	0.8
328401	510	mir-196-2	0.8
328412	521	mir-218-1	1.2
328414	523	mir-124a-2	0.9
328418	527	mir-144	1.0
328419	528	mir-221	0.7
328430	539	mir-129-2	1.3
328432	541	hypothetical miRNA-075	0.6
328434	543	mir-15b	0.8
328640	549	let-7e	0.9
328641	550	hypothetical miRNA-083	1.1
328642	551	let-7c	1.0
328644	553	mir-145	0.7
328650	559	mir-133a-1	0.8
328658	567	hypothetical miRNA-101	1.2
328690	599	mir-204	0.8
328693	602	mir-213	1.0
328697	606	mir-181b	1.0

From these data, it is evident that SEQ ID NOs. 480, 509, 601, 490, 524, 557, 506, 565, 530, 605, 492, 561, 511, 555, 483, 502, 535, 562, 544, 519, 516, 554, 496, 567, 521, 539, 488,

498, and 550 induce apoptosis in T47D cells, while SEQ ID NOs. 500, 486, 518, 532, 534, 566, 603, 609, 485, 495, 520, 522, 536, 547, 548, 564, 598, 604, 503, 505, 510, 528, 541, 543, 553, 559, and 599 prevent or have a protective effect from apoptosis in the same system.

Example 12: Oligomeric compounds targeting the mir-30a pri-miRNA structure

In one embodiment of the invention, oligomeric compounds targeting the hairpin structure of mir-30a pri-miRNA were designed and tested for their effects on miRNA signaling in 293T cells (American Type Culture Collection (Manassas, VA)).

A synthetic DNA fragment comprised of four tandem repeats of the target site for mir-30a was cloned into the vector pGL3-C (purchased from Promega Corp., Madison WI) at the unique XbaI site (pGL3C-M30-4X). This places the target site in the 3'UTR of the luciferase reporter vector. An oligomeric compound mimicking the mir-30a pri-miRNA (**AATTTAATACGACTCACTATAGG**GCGACTGTAAACATCCTCGACTGGAAGCTGTG AAGCCACAGATGGGCTTTCAGTCGGATGTTTGCAGCTGC, herein incorporated as SEQ ID NO: 1749) was *in vitro* transcribed using T7 RNA polymerase and a DNA template produced by PCR (the T7 promoter is shown in bold).

On the day prior to the experiment 24-well plates were seeded with 293T cells at 50% confluency. The following morning cells were treated with oligomeric compounds targeted to the mir-30a pri-miRNA mimic. The oligomeric compounds used in this study are shown in Table 13. All of the compounds are 20 nucleobases in length having either a phosphorothioate backbone throughout (PS) or a phosphodiester backbone throughout (PO). As designated in the table, ISIS 328076, 328078, 328081, 328084, 328086, 328088 are chimeric oligomeric compounds ("gapmers") 20 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide "wings." The wings are composed of 2'-methoxyethoxy (2'-MOE) nucleotides. All cytidine residues are 5-methylcytidines. The remaining compounds in the table have 2'-methoxyethoxy (MOE) nucleotides throughout with either a phosphorothioate (PS) or phosphodiester (PO) internucleoside linkages.

If the compound targeted the pre-loop of the mir-30a pri-miRNA structure, that designation is also noted in the table.

Table 13

Oligomeric compounds targeting the mir-30a pri-miRNA

Isis Number	Sequence	Chemistry	SEQ ID NO
328075	GCTTCACAGCTTCCAGTCGA	(PS/MOE)	740

328076	GCTTCACAGCTTCCAGTCGA	(PS/MOE 5-10-5 gapmer)	740
328077	CCCATCTGTGGCTTCACAGC	(PS/MOE); pre-loop	741
328078	CCCATCTGTGGCTTCACAGC	(PS/MOE 5-10-5 gapmer); pre-loop	741
328079	CCCATCTGTGGCTTCACAGC	(PO/MOE); pre-loop	741
328080	TGAAAGCCCATCTGTGGCTT	(PS/MOE); pre-loop	742
328081	TGAAAGCCCATCTGTGGCTT	(PS/MOE 5-10-5 gapmer); pre-loop	742
328082	TGAAAGCCCATCTGTGGCTT	(PO/MOE); pre-loop	742
328083	GCAGCTGCAAACATCCGACT	(PS/MOE)	743
328084	GCAGCTGCAAACATCCGACT	(PS/MOE 5-10-5 gapmer)	743
328085	CATCTGTGGCTTCACAGCTT	(PS/MOE)	744
328086	CATCTGTGGCTTCACAGCTT	(PS/MOE 5-10-5 gapmer)	744
328087	AAGCCCATCTGTGGCTTCAC	(PS/MOE)	745
328088	AAGCCCATCTGTGGCTTCAC	(PS/MOE 5-10-5 gapmer)	745

Cells were washed once with PBS then oligomeric compounds were added to triplicate wells at 150 nM in OPTI-MEM™ media and 4.5 µl/ml LIPOFECTIN™ reagent (Invitrogen Corporation, Carlsbad, CA). After 3 hours, the media was removed, and the cells were treated with the mir-30a pri-miRNA mimic at 200nM in OPTI-MEM™ with 6 µl/ml LIPOFECTIN™ reagent. After 3 hours the media was removed from the cells. The reporter plasmid, pGL3C-M30-4X, was then transfected using SuperFect reagent. 20 µg of pGL3C-M30-4X and 2 µg of pRL-CMV, a plasmid expressing *Renilla* luciferase, were suspended in 600 µl of serum-free DMEM to which 120 µl of Superfect was added. After a 5 minute incubation, 6 mls of DMEM + 10% FCS was added. 125 µl of the plasmid/SuperFect suspension was added to each well. After a 2 hour incubation cells were washed and fresh growth media added. Cells were incubated overnight.

The following morning the media was removed and the cells were lysed in 120 µl passive lysis buffer (PLB; Promega). 40 µl of the lysate was then assayed for *Photinus (PL)* and *Renilla* (RL) luciferases using a Dual Luciferase Assay kit (Promega) according to the manufacturer's protocol. The results below are given as percent pGL3C-M30-4X expression (PL) normalized to pRL-CMV expression (RL). The 20-nucleobase oligonucleotide random-mer ISIS Number 29848 was used as a negative control. The data are shown in Table 14.

Table 14

Effects of oligomeric compounds targeting the mir-30a pri-miRNA on reporter gene expression

SEQ ID NO	ISIS Number	percent control luciferase expression
N/A	Untreated control	100
N/A	Mir-30a pri-miRNA only	62
737	29848 control added after mir-30a pri-miRNA	63
292	327874	66
740	328075	55

740	328076	57
741	328077	70
741	328078	63
742	328080	72
742	328081	80
743	328084	75
744	328085	72
744	328086	95
745	328087	83
745	328088	107

Upon adminstration of the mir-30a pri-miRNA mimic, the pri-miRNA is believed to be processed in the cell by the endogenous Drosha RNase III enzyme into a pre-miRNA, which is then processed by human Dicer into a mature miRNA, which is then able to hybridize to the target site, thus effectively reducing luciferase reporter expression.

Upon treatment of the system with the oligomeric compounds targeting the mir-30a pri-miRNA, the processing and/or production of the mir-30a mature miRNA is inhibited, and the mir-30a miRNA is no longer able to bind its target site, thus allowing luciferase reporter expression to increase.

Cells treated with mir-30a pri-miRNA mimic show an approximately 38% reduction in luciferase expression compared to the untreated controls. Treatment with ISIS 328086, 328087 and 328088 had the most dramatic effect in reversing the mir-30a miRNA-mediated silencing, restoring luciferase reporter expression to near control levels. Thus, it was demonstrated that the oligomeric compound mimicking the mir-30a pri-miRNA silences luciferase activity from the reporter vector, and that oligomeric compounds targeting the mir-30a pri-miRNA can inhibit its silencing activity, possibly by interfering with its processing into the pre-miRNA or mature miRNA molecules.

ISIS 328085 to ISIS 328088 were designed to target the mir-30a pri-miRNA as pseudo half-knot compounds. Methods for the preparation of pseudo half-knot compounds are disclosed in US Patent 5,512,438 which is incorporated herein by reference. This motif has been used to disrupt the structure of regulatory RNA stem loops in larger viral genomic structures. (Ecker et al, *Science*. **1992**; *257*:958-61). However, this is the first example of the pseudo half-knot motif being used to regulate a small non-coding RNA, more specifically a miRNA such as those disclosed herein. It is also the first demonstration of apoptotic modulation in a cell by pseudo half-knot structured oligomeric compounds.

Example 13: Effects of oligomeric compounds targeting miRNAs on expression of adipocyte differentiation markers

The effect of several oligomeric compounds of the present invention targeting miRNAs on the expression of markers of cellular differentiation was examined in preadipocytes.

One of the hallmarks of cellular differentiation is the upregulation of gene expression. During adipocyte differentiation, the gene expression patterns in adipocytes change considerably. An excessive recruitment and differentiation of preadipocytes into mature adipocytes is a characteristic of human obesity, which is a strong risk factor for type 2 diabetes, hypertension, atherosclerosis, cardiovascular disease, and certain cancers. Some genes known to be upregulated during adipocyte differentiation include hormone-sensitive lipase (HSL), adipocyte lipid binding protein (aP2), glucose transporter 4 (Glut4), and PPARγ (Peroxisome proliferator-activated receptor gamma). These genes play important roles in the uptake of glucose and the metabolism and utilization of fats. For example, HSL is involved in the mobilization of fatty acids from adipose tissue into the bloodstream; studies suggest that increased free fatty acid levels are one of the causative factors in type 2 diabetes. aP2 is believed to play a role in athersclerosis. Glut4 is important in insulin signaling. PPARγ is believed to be involved in adipocyte differentiation, insulin sensitivity, and colonic tumor development.

Leptin is also a marker for differentiated adipocytes. In the adipocyte assay, leptin secretion into the media above the differentiated adipocytes was measured by protein ELISA. Cell growth, transfection and differentiation procedures were carried out as described for the Triglyceride accumulation assay (see below). On day nine post-transfection, 96-well plates were coated with a monoclonal antibody to human leptin (R&D Systems, Minneapolis, MN) and left at 4°C overnight. The plates were blocked with bovine serum albumin (BSA), and a dilution of the media was incubated in the plate at RT for 2 hours. After washing to remove unbound components, a second monoclonal antibody to human leptin (conjugated with biotin) was added. The plate was then incubated with strepavidin-conjugated horseradish peroxidase (HRP) and enzyme levels are determined by incubation with 3, 3', 5, 5'-Tetramethlybenzidine, which turns blue when cleaved by HRP. The OD_{450} was read for each well, where the dye absorbance is proportional to the leptin concentration in the cell lysate. Results are expressed as a percent ± standard deviation relative to transfectant-only controls.

An increase in triglyceride content is another well-established marker for adipocyte differentiation. The triglyceride accumulation assay measures the synthesis of triglyceride by adipocytes. Triglyceride accumulation was measured using the Infinity™ Triglyceride reagent kit (Sigma-Aldrich, St. Louis, MO). Human white preadipocytes (Zen-Bio Inc., Research Triangle Park, NC) were grown in preadipocyte media (ZenBio Inc.). One day before transfection, 96-well plates were seeded with 3000 cells/well. Cells were transfected according

to standard published procedures with 250nM oligomeric compound in LIPOFECTIN™ (Invitrogen Corporation, Carlsbad, CA) (Monia et al., *J. Biol. Chem.* **1993** *268*(19):14514-22). Oligomeric compounds were tested in triplicate on each 96-well plate, and the effects of TNF-α, a positive drug control that inhibits adipocyte differentiation, were also measured in triplicate. Negative and transfectant-only controls may be measured up to six times per plate. After the cells have reached confluence (approximately three days), they were exposed to differentiation media (Zen-Bio, Inc.) containing a PPAR-γ agonist, IBMX, dexamethasone, and insulin for three days. Cells were then fed adipocyte media (Zen-Bio, Inc.), which was replaced at 2 to 3 day intervals. On day nine post-transfection, cells were washed and lysed at room temperature, and the triglyceride assay reagent was added. Triglyceride accumulation was measured based on the amount of glycerol liberated from triglycerides by the enzyme lipoprotein lipase. Liberated glycerol is phosphorylated by glycerol kinase, and hydrogen peroxide is generated during the oxidation of glycerol-1-phosphate to dihydroxyacetone phosphate by glycerol phosphate oxidase. Horseradish peroxidase (HRP) uses H_2O_2 to oxidize 4-aminoantipyrine and 3,5 dichloro-2-hydroxybenzene sulfonate to produce a red-colored dye. Dye absorbance, which is proportional to the concentration of glycerol, was measured at 515nm using an UV spectrophotometer. Glycerol concentration was calculated from a standard curve for each assay, and data were normalized to total cellular protein as determined by a Bradford assay (Bio-Rad Laboratories, Hercules, CA). Results are expressed as a percent ± standard deviation relative to transfectant-only control.

For assaying adipocyte differentiation, expression of the four hallmark genes, HSL, aP2, Glut4, and PPARγ, as well as triglyceride (TG) accumulation and leptin secretion were measured in adipocytes transfected with the uniform 2'-MOE phosphorothioate (PS) oligomeric compounds previously described. Cells are lysed on day nine post-transfection, in a guanadinium-containing buffer and total RNA is harvested. Real-time PCR is performed (Applied Biosystems, Prism 7700) on the total RNA using the following primer/probe sets for the adipocyte differentiation hallmark genes: (aP2): forward 5'-GGTGGTGGAATGCGTCATG-3' (SEQ ID NO: 746), reverse 5'-CAACGTCCCTTGGCTTATGC-3' (SEQ ID NO: 747), probe 5'-FAM-AAGGCGTCACTTCCACGAGAGTTTATGAGA-TAMRA-3' (SEQ ID NO: 748); (Glut4): forward 5'-GGCCTCCGCAGGTTCTG-3' (SEQ ID NO: 749), reverse 5'-TTCGGAGCCTATCTGTTGGAA-3' (SEQ ID NO: 750), probe 5'-FAM-TCCAGGCCGGAGTCAGAGACTCCA-TAMRA-3' (SEQ ID NO: 751); (HSL): forward 5'-ACCTGCGCACAATGACACA-3' (SEQ ID NO: 752), reverse 5'-

TGGCTCGAGAAGAAGGCTATG-3' (SEQ ID NO: 753), probe 5'-FAM-CCTCCGCCAGAGTCACCAGCG-TAMRA-3' (SEQ ID NO: 754); (PPAR-γ): forward 5'-AAATATCAGTGTGAATTACAGCAAACC-3' (SEQ ID NO: 755), reverse 5'-GGAATCGCTTTCTGGGTCAA-3' (SEQ ID NO: 756), probe 5'-FAM-TGCTGTTATGGGTGAAACTCTGGGAGATTCT-TAMRA-3' (SEQ ID NO: 757). The amount of total RNA in each sample is determined using a Ribogreen Assay (Molecular Probes, Eugene, OR), and expression levels of the adipocyte differentiation hallmark genes were normalized to total RNA. Leptin protein and triglyceride levels as well as mRNA levels for each of the four adipocyte differentiation hallmark genes are expressed relative to control levels (control = treatment with ISIS-29848 (SEQ ID NO: 737)). Results of two experiments are shown in Tables 15 and 16.

Table 15

Effects of oligomeric compounds targeting miRNAs on expression of adipocyte differentiation markers

ISIS Number	SEQ ID NO	TG	AP2	HSL	Glut4	PPAR gamma
327876	294	0.47	0.75	0.47	0.36	0.57
327878	296	0.65	0.85	0.93	0.69	0.97
327880	298	0.52	0.97	0.80	1.11	0.53
327888	306	0.98	1.18	1.38	1.37	1.36
327889	307	0.47	0.69	0.59	0.55	0.71
327890	308	0.92	0.91	0.86	1.10	1.18
327892	310	0.42	0.31	0.25	0.07	0.32
327901	319	0.54	0.42	0.33	0.19	0.30
327903	321	1.20	1.15	1.23	1.72	1.19
327905	323	0.69	1.14	1.11	0.84	0.54
327913	331	0.59	0.99	0.92	0.84	0.72
327919	337	0.58	0.79	0.57	0.32	0.52
327922	340	1.09	0.99	0.95	1.75	1.37
327925	343	0.72	0.77	0.78	1.99	0.60
327933	351	1.48	1.46	1.35	2.52	1.52
327934	352	0.99	1.20	1.02	1.22	0.97
327939	357	0.92	1.08	1.21	0.87	0.83
327941	359	1.31	1.78	1.73	2.07	0.80
327954	372	0.58	0.95	1.03	0.92	0.73

Table 16

Effects of oligomeric compounds targeting miRNAs on expression of adipocyte differentiation markers

ISIS Number	SEQ ID NO	TG	Leptin	AP2	HSL	Glut4	PPAR gamma
327888	306	0.44	1.38	0.47	0.50	0.17	0.66
327889	307	0.46	1.05	0.57	0.54	0.46	0.82
327890	308	0.61	1.36	0.69	0.67	0.67	0.94
327893	311	0.95	1.14	0.97	0.85	1.47	1.03

327901	319	0.53	1.02	0.47	0.47	0.29	0.72
327903	321	0.58	1.61	0.92	0.80	1.12	0.98
327905	323	0.58	1.62	0.68	0.69	0.40	0.83
327919	337	0.40	1.44	0.48	0.37	0.18	0.57
327922	340	0.43	1.25	0.75	0.72	0.43	0.80
327925	343	0.63	1.40	0.77	0.75	0.61	0.83
327926	344	1.06	1.47	0.85	0.82	1.10	0.93
327930	348	0.97	0.95	0.86	0.89	1.01	0.98
327931	349	1.11	1.12	1.00	0.99	1.37	1.56
327934	352	0.62	1.25	0.66	0.64	0.44	0.72
327938	356	1.05	1.35	0.86	0.85	0.80	0.90
327939	357	0.59	2.67	0.69	0.63	0.30	0.70
327941	359	0.42	0.54	0.88	0.81	0.44	0.86
327942	360	0.85	2.03	0.82	0.79	0.66	0.87
327955	373	0.81	1.22	0.74	0.82	0.45	0.92
327967	385	0.90	1.22	0.86	0.97	0.56	0.89

From these data, values above 1.0 for triglyceride accumulation (column "TG" in the tables) indicate that the compound has the ability to stimulate triglyceride accumulation, whereas values at or below 1.0 indicate that the compound inhibits triglyceride accumulation. With respect to leptin secretion (column "Leptin" in the tables), values above 1.0 indicate that the compound has the ability to stimulate secretion of the leptin hormone, and values at or below 1.0 indicate that the compound has the ability to inhibit secretion of leptin. With respect to the four adipocyte differentiation hallmark genes (columns "AP2," "HSL," "Glut4," and "PPAR gamma" in the tables), values above 1.0 indicate induction of cell differentiation, whereas values at or below 1.0 indicate that the compound inhibits differentiation.

Several compounds were found to have remarkable effects. For example, the oligomeric compounds ISIS Number 327889 (SEQ ID NO: 307), targeted to mir-23b; ISIS Number 327892 (SEQ ID NO: 310), targeted to mir-131-1, mir-131-2 and mir-131-3 (also known as mir-9); ISIS Number 327942 (SEQ ID NO: 360) targeted to mir-141 and ISIS Number 327901 (SEQ ID NO: 319), targeted to mir-143 were shown to significantly reduce the expression levels of 5 of 6 markers of adipocyte differentiation (excepting leptin levels), indicating that these oligomeric compounds have the ability to block adipocyte differentiation. Therefore, these oligomeric compounds may be useful as pharmaceutical agents with applications in the treatment, attenuation or prevention of obesity, hyperlipidemia, atherosclerosis, atherogenesis, diabetes, hypertension, or other metabolic diseases as well as having potential applications in the maintenance of the pluripotent phenotype of stem or precursor cells.

The compound ISIS Number 327939 (SEQ ID NO: 357), targeted to mir-125b-1, for example, produced surprising results in that it demonstrates a significant increase in leptin secretion but a concomitant decrease in triglyeride accumulation and a decrease in the expression of all four adipocyte differentiation hallmark genes, indicating that this oligomeric compound

may be useful as a pharmaceutic agent in the treatment of obesity, as well as having applications in other metabolic diseases.

The oligomeric compound ISIS Number 327931 (SEQ ID NO: 349), targeted to let-7c is an example of a compound which demonstrates an increase in four out of six markers of adipocyte differentiation, including a significant increase in the expression of PPAR-γ. This oligomeric compound may be useful as a pharmaceutical agent in the treatment of diseases in which the induction of cell differentiation is desirable.

The oligomeric compound ISIS Number 327933 (SEQ ID NO: 351), targeted to mir-145 is an example of a compound which demonstrates an increase in all six markers of adipocyte differentiation. This oligomeric compound may be useful as a pharmaceutical agent in the treatment of diseases in which the induction of adipocyte differentiation is desirable, such as anorexia, or for conditions or injuries in which the induction of cellular differentiation is desireable, such as Alzheimers disease or central nervous system injury, in which regeneration of neural tissue (such as from pluripotent stem cells) would be beneficial. Furthermore, this oligomeric compound may be useful in the treatment, attenuation or prevention of diseases in which it is desireable to induce cellular differentiation and/or quiescence, for example in the treatment of hyperproliferative disorders such as cancer.

In some embodiments, differentiating adipocytes were treated with uniform 2'-MOE phosphorothioate oligomeric compounds according to the methods described above, and the expression of the four hallmark genes, HSL, aP2, Glut4, and PPARγ, as well as triglyceride (TG) accumulation were measured. TG levels as well as mRNA levels for each of the four adipocyte differentiation hallmark genes are expressed as a percentage of control levels (control = treatment with ISIS 342673; AGACTAGCGGTATCTTTATCCC; herein incorporated as SEQ ID NO: 758), a uniform 2'-MOE phosphorothioate oligomeric compound containing 15 mismatches with respect to the mature mir-143 miRNA). Undifferentiated adipocytes were also compared as a negative control. As a positive control, differentiating adipocytes were treated with ISIS 105990 (AGCAAAAGATCAATCCGTTA; herein incorporated as SEQ ID NO: 759), a 5-10-5 gapmer oligomeric compound targeting the PPAR-gamma mRNA, previously demonstrated to inhibit adipocyte differentiation. The effects of TNF-α, also known to inhibit adipocyte differentiation, were also measured. Results of these experiments are shown in Tables 17 and 18.

Table 17

Effects of oligomeric compounds targeting miRNAs on expression of adipocyte differentiation markers

ISIS Number	SEQ ID NO	TG	AP2	HSL	Glut4	PPAR gamma
Untreated control	N/A	88.5	87.8	88.6	102.7	94.9
105990	759	28.2	51.6	49.2	59.5	51.8
342673	758	100.0	100.0	100.0	100.0	100.0
TNF-alpha	N/A	10.0	5.5	0.7	0.5	18.8
Undiff. adipocytes	N/A	2.7	0.0	0.3	0.1	9.2
328116	418	82.1	87.7	75.8	75.2	78.4
328117	419	55.0	65.4	61.7	68.1	64.1
328118	420	69.3	92.7	85.3	76.6	80.2
328119	421	90.2	99.9	98.5	95.2	82.7
328120	422	82.7	81.0	77.7	94.8	70.5
328121	423	134.8	127.0	126.0	140.8	103.6
328122	424	78.9	79.3	72.7	85.9	77.8
328123	425	120.8	106.7	85.4	162.4	74.7
328124	426	99.1	101.8	103.6	122.7	90.4
328125	427	81.7	86.9	75.8	99.5	76.1
328126	428	98.9	90.9	83.2	100.7	75.0
328127	429	74.5	86.9	89.7	80.8	77.6
328128	430	98.7	100.7	94.1	101.9	84.0
328129	431	53.8	67.6	56.5	60.0	71.8
328130	432	122.4	86.6	76.5	83.8	99.4
328131	433	89.1	95.4	81.8	103.6	88.2
328132	434	114.1	90.2	73.7	72.1	90.0
328133	435	61.2	69.5	63.0	91.9	63.8
328134	436	85.7	80.1	74.7	88.3	78.4
328135	437	63.6	80.6	76.7	90.3	70.0
328136	438	47.0	73.0	65.0	66.7	72.7
328137	439	83.2	99.6	86.3	88.5	85.7
328138	440	100.6	85.3	89.8	86.8	83.8
328139	441	89.1	98.3	92.6	106.3	115.0

Table 18

Effects of oligomeric compounds targeting miRNAs on expression of adipocyte differentiation markers

ISIS #	SEQ ID NO	TG	AP2	HSL	Glut4	PPAR gamma
Untreated control	N/A	102.2	90.8	94.9	117.8	103.3
105990	759	32.8	49.8	52.0	68.1	60.1
342673	758	100	100	100	100	100
TNF-alpha	N/A	14.5	9.6	3.1	1.9	27.9
Undiff. adipocytes	N/A	2.8	0.0	1.4	0.3	10.7
327912	330	107.4	90.1	90.6	89.0	76.9
327969	387	46.0	59.8	66.4	60.6	69.2
328099	401	93.9	85.9	88.4	86.8	81.9
328100	402	71.5	61.9	72.0	74.2	66.7
328101	403	108.6	83.2	91.8	84.7	79.3
328102	404	95.9	87.9	97.0	79.2	93.7
328103	405	110.2	83.2	82.5	94.3	74.3
328104	406	122.6	102.2	98.2	119.1	90.4
328105	407	93.1	88.2	94.2	94.2	93.3
328106	408	90.5	88.8	94.9	105.7	90.7

328107	409	66.7	67.5	61.0	72.5	79.3
328108	410	89.6	83.7	90.1	94.9	84.0
328109	411	84.9	84.9	86.9	106.6	96.1
328110	412	97.7	93.3	91.0	104.7	91.2
328111	413	101.9	71.5	69.5	59.6	74.9
328112	414	98.1	99.1	101.2	122.5	102.4
328113	415	80.8	84.5	90.6	99.9	93.8
328114	416	117.3	94.4	93.3	114.9	89.3
328115	417	108.7	80.0	89.0	132.0	95.8
341803	760	85.9	77.3	75.5	86.8	71.2
341804	761	60.9	70.8	71.6	73.6	74.1
341805	762	78.1	81.9	81.8	88.2	80.4
341806	763	83.2	75.8	73.4	69.4	72.6
341807	764	114.1	74.8	96.8	119.5	86.2

Several compounds were found to have remarkable effects. For example, the oligomeric compounds ISIS Number 328117 (SEQ ID NO: 419), targeted to hypothetical miRNA-144, ISIS Number 328129 (SEQ ID NO: 431), targeted to hypothetical miRNA-173, ISIS Number 328136 (SEQ ID NO: 438), targeted to hypothetical miRNA-181, and ISIS Number 327969 (SEQ ID NO: 387), targeted to mir-182, were each shown to reduce the expression levels of triglycerides by at least 50%, and treatment with ISIS 328117, 328129, or 328136 also each resulted in a reduction of expression of the other four hallmark genes, indicating that these oligomeric compounds targeted to hypothetical miRNA-144, hypothetical miRNA-173, hypothetical miRNA-181, and mir-182, may be useful as therapeutic agents with applications in the treatment, attenuation or prevention of obesity, hyperlipidemia, atherosclerosis, atherogenesis, diabetes, hypertension, or other metabolic diseases.

The oligomeric compound ISIS Number 328121 (SEQ ID NO: 423), targeted to hypothetical miRNA-161 is an example of a compound which stimulates an increase in all five markers of adipocyte differentiation. This oligomeric compound may be useful as a pharmaceutical agent in the treatment of diseases in which the induction of adipocyte differentiation is desirable, such as anorexia, or for conditions or injuries in which the induction of cellular differentiation is desireable, such as Alzheimers disease or central nervous system injury, in which regeneration of neural tissue would be beneficial. Furthermore, this oligomeric compound may be useful in the treatment, attenuation or prevention of diseases in which it is desireable to induce cellular differentiation and/or quiescence, for example in the treatment of hyperproliferative disorders such as cancer.

Example 14: Expression of mir-143 in human tissues and cell lines

Total RNA from spleen, kidney, testicle, heart and liver tissues as well as total RNA from human promyelocytic leukemia HL-60 cells, human embryonic kidney 293 (HEK293)

cells, and T47D human breast carcinoma cells was purchased from Ambion, Inc. (Austin, TX). RNA from preadipocytes and differentiated adipocytes was purchased from Zen-Bio, Inc. (Research Triangle Park, NC). RNA was prepared from the HeLa, NT2, T-24, and A549 cell lines cultured as described above, using the following protocol: cell monolayers were washed twice with cold PBS, and cells were lysed in 1 mL TRIZOL™ (Invitrogen) and total RNA prepared using the manufacturer's recommended protocols.

Fifteen to twenty micrograms of total RNA was fractionated by electrophoresis through 10% acrylamide urea gels using a TBE buffer system (Invitrogen). RNA was transferred from the gel to HYBOND™-N+ nylon membranes (Amersham Pharmacia Biotech, Piscataway, NJ) by electroblotting in an Xcell SureLock™ Minicell (Invitrogen). Membranes were fixed by UV cross-linking using a STRATALINKER™ UV Crosslinker 2400 (Stratagene, Inc, La Jolla, CA) and then probed using Rapid Hyb buffer solution (Amersham) using manufacturer's recommendations for oligonucleotide probes.

To detect mir-143, a target specific DNA oligonucleotide probe with the sequence TGAGCTACAGTGCTTCATCTCA (SEQ ID NO: 319) was synthesized by IDT (Coralville, IA). The oligo probe was 5' end-labeled with T4 polynucleotide kinase with (γ-^{32}P) ATP (Promega). To normalize for variations in loading and transfer efficiency membranes can be stripped and probed for U6 RNA. Hybridized membranes were visualized and quantitated using a Storm 860 PHOSPHORIMAGER™ and IMAGEQUANT™ Software V3.3 (Molecular Dynamics, Sunnyvale, CA).

Using this probe, the mir-143 miRNA was found to be most highly expressed in human heart, thymus and kidney, and was also expressed to a lesser extent in lung, spleen, liver, and brain tissues. For example, as compared to expression levels in liver, mir-143 was expressed approximately 24-fold higher in heart, 17-fold higher in thymus, and 8-fold higher in kidney.

The mir-143 miRNA was also found to be expressed in adipocytes and preadipocytes, and levels of mir-143 were found to be dramatically upregulated in differentiated adipocytes as compared to preadipocytes, indicating that this miRNA may be important in adipocyte differentiation. These data, taken together with the finding that the oligomeric compound, ISIS Number 327901 (SEQ ID NO: 319), targeted to mir-143, was shown to inhibit the adipocyte differentiation markers (described above, Example 13), supports the conclusion that mir-143 is involved in cellular differentiation pathways.

Example 15: Effects of oligomeric compounds targeting miRNAs on apoptosis in the caspase assay in preadipocytes

The effect of oligomeric compounds of the present invention targeting miRNAs was examined in preadipocytes (Zen-Bio, Inc., Research Triangle Park, NC) using the fluorometric caspase assay previously described in Example 11. The oligonucleotide random-mer, ISIS-29848 (SEQ ID NO: 737) was used as a negative control, and ISIS-148715 (SEQ ID NO: 738), targeting the human Jagged2 mRNA, known to induce apoptosis when inhibited, was used as a positive control. The measurement obtained from the untreated control cells is designated as 100% activity and was set equal to 1.0. Results are shown in Table 19.

Table 19

Effects of targeting miRNAs on apoptosis in preadipocytes

ISIS Number	SEQ ID NO.	Pri-miRNA	Fold Increase over UTC
UTC Untreated control	N/A	N/A	1.0
ISIS-29848 n-mer	737	N/A	1.2
ISIS-148715 Positive control	738	Jagged2	36.9
327888	306	mir-108-1	1.1
327889	307	mir-23b	1.1
327890	308	let-7i	1.3
327893	311	let-7b	1.3
327901	319	mir-143	2.0
327903	321	let-7a-3	1.6
327905	323	mir-205	1.5
327919	337	mir-221	1.3
327922	340	mir-19b-2	1.0
327925	343	mir-133b	2.0
327926	344	let-7d	1.8
327930	348	let-7e	1.4
327931	349	let-7c	1.5
327934	352	mir-213	2.0
327938	356	mir-98	1.0
327939	357	mir-125b-1	2.2
327941	359	mir-181b	1.3
327942	360	mir-141	1.0
327955	373	mir-130b	4.3
327967	385	let-7g	1.5

From these data, it is evident that the oligomeric compounds of the present invention generally do not induce the activity of caspases involved in apoptotic pathways in preadipocytes. In particular, the oligomeric compound targeting mir-143, ISIS Number 327901 (SEQ ID NO: 319), does not result in a significant increase in caspase activity as compared to the Jagged2 positive control. Taken together with the results from the adipocyte differentiation assay (Example 13) and the expression analysis of mir-143 (Example 14), these data suggest that the

mir-143 miRNA plays a role in stimulating cellular differentiation, employing pathways other than the caspase cascades activated during apoptosis.

It was recently reported that bone marrow cells may contribute to the pathogenesis of vascular diseases, and that cell differentiation appears to be important in models of postangioplasty restenosis, graft vasculopathy, and hyperlipidemia-induced atherosclerosis. Bone marrow cells have the potential to give rise to vascular progenitor cells that home in on damaged vessels and differentiate into smooth muscle cells or endothelial cells, thereby contributing to vascular repair, remodeling, and lesion formation (Sata, M. *Trends Cardiovasc Med.* **2003** 13(6):249-53). Thus, the ability to modulate cell differentiation may provide the basis for the development of new therapeutic strategies for vascular diseases, targeting mobilization, homing, differentiation, and proliferation of circulating vascular progenitor cells.

Example 16: Comparison of effects of oligomeric compounds targeting the mir-143 pri-miRNA or mature mir-143 miRNA on adipocyte differentiation

Two oligomeric compounds targeting the mature mir-143 miRNA and two oligomeric compounds targeting the 110-nucleotide mir-143 pri-miRNA were compared for their effects on adipocyte differentiation using the same adipocyte differentiation assay as described in Example 13.

The oligomeric compound, ISIS Number 327901 (SEQ ID NO: 319), 22-nucleotides in length, targets the mature mir-143 miRNA and is composed of 2'-methoxyethoxy (2'-MOE) nucleotides and phosphorothioate (P=S) internucleoside (backbone) linkages throughout. The oligomeric compound ISIS Number 338664 (CAGACTCCCAACTGACCAGA; SEQ ID NO: 491) is also a uniform 2'-MOE oligonucleotide, which is designed to target the mir-143 pri-miRNA. Another oligomeric compound targeting the mir-143 pri-miRNA, ISIS Number 328382 (SEQ ID NO: 491) is a chimeric oligonucleotide, 20 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide "wings" having 2'-MOE substituents in the wing nucleosides (a "5-10-5 gapmer"), and ISIS Number 340927 (TGAGCTACAGTGCTTCATCTCA; SEQ ID NO: 319) is a 5-10-7 gapmer designed to target mature mir-143. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines. The effect of these oligomeric compounds targeting the mir-143 miRNA and the mir-143 pri-miRNA on expression of the 5 hallmark genes indicating cellular differentiation was examined in preadipocytes using the same methods described in Example 13. Results are shown in Table 20.

Table 20

Comparison of uniform 2'-MOE and chimeric oligomeric compounds targeting the mir-143 miRNA and pri-miRNAs on expression of adipocyte differentiation markers

ISIS Number	SEQ ID NO	TG	AP2	HSL	Glut4	PPAR gamma
327901	319	0.54	0.42	0.33	0.19	0.30
328382	491	0.72	0.89	0.75	0.85	0.96
338664	491	1.42	1.01	0.76	1.81	0.86
340927	319	0.65	0.77	0.73	0.54	0.36

From these data, it was observed that while the gapmer oligomeric compound targeting the mature mir-143 (ISIS Number 340972) results in reduced expression of the adipocyte differentiation markers, the uniform 2'-MOE oligomeric compound targeting mature mir-143 (ISIS Number 327901) was more effective. For the oligomeric compounds targeting the mir-143 pri-miRNA, the gapmer compound (ISIS Number 328382) appeared to be more effective in blocking adipocyte differentiation than was the uniform 2'-MOE oligomeric compound (ISIS Number 338664).

Dose responsiveness:

In one embodiment, the oligomeric compound ISIS Number 327901 (SEQ ID NO: 319) targeting mature mir-143 was selected for additional dose response studies in the adipocyte differentiation assay. Differentiating adipocytes (at day 10 post-induction of differentiation) were treated with 50, 100, 200, and 300 nM ISIS 327901, or the scrambled control ISIS Number 342673 (SEQ ID NO: 758) containing 15 mismatches with respect to the mature mir-143 miRNA. ISIS Numbers 327901 and 342673 are uniform 2'-MOE phosphorothioate oligomeric compounds 22 nucleotides in length. Differentiating adipocytes treated with ISIS Number 29848 (SEQ ID NO: 737) served as the negative control to which the data were normalized. Differentiating adipocytes treated with ISIS 105990 (SEQ ID NO: 759), a 5-10-5 gapmer oligomeric compound targeting the PPAR-gamma mRNA which has been demonstrated previously to inhibit adipocyte differentiation, served as the positive control. Triglyceride levels as well as mRNA levels for each of the four adipocyte differentiation hallmark genes (PPAR-gamma, aP2, HSL, and GLUT4) were measured 24 hours after treatment as described above. Untreated cells were compared to cells treated with oligomeric compounds, and results of these dose response studies are shown in Table 21, where levels of the markers is expressed as a percentage of untreated control (% UTC) levels. Where present, "N.D." indicates "no data."

Table 21

Effects of oligomeric compounds targeting mir-143 on expression of adipocyte differentiation markers

Hallmark Measured:	Isis #:	% UTC			
		Dose of oligomeric compound			
		50 nM	100 nM	200 nM	300 nM
Triglycerides	342673 negative control	94.2	105.3	98.3	108.2
	105990 positive control	N.D.	N.D.	N.D.	16.6
	327901	85.3	68.9	34.0	23.0
PPAR-gamma mRNA	342673 negative control	77.5	89.9	94.6	85.8
	105990 positive control	N.D.	N.D.	N.D.	43.9
	327901	74.6	70.8	51.8	39.3
AP2 mRNA	342673 negative control	82.4	90.3	81.1	70.9
	105990 positive control	N.D.	N.D.	N.D.	17.9
	327901	78.3	64.6	39.0	22.4
HSL mRNA	342673 negative control	92.0	95.6	97.3	85.2
	105990 positive control	N.D.	N.D.	N.D.	7.4
	327901	89.5	73.5	40.2	11.9
GLUT4 mRNA	342673 negative control	94.9	90.7	97.6	102.7
	105990 positive control	N.D.	N.D.	N.D.	11.8
	327901	74.2	49.7	32.8	17.4

From these data, it was observed that treatment of differentiating adipocytes with the uniform 2'-MOE oligomeric compound, ISIS Number 327901 targeting mir-143 results in a dose responsive reduction of expression of all five markers of differentiation. Thus, this oligomeric compound may be useful in the treatment of diseases associated with increased expression of these hallmark genes, such as obesity, hyperlipidemia, atherosclerosis, atherogenesis, diabetes, hypertension, or other metabolic diseases as well as having potential applications in the maintenance of the pluripotent phenotype of stem or precursor cells.

Example 17: Human Let 7 homologs

Let-7 is one of the two miRNAs originally identified in *C. elegans* as an antisense translational repressor of messenger RNAs encoding key developmental timing regulators in nematode larva. Several genes predicted to encode let-7-like miRNAs have been identified in a wide variety of species, and these let-7-like homologs are believed to control temporal

transitions during development across animal phylogeny. Oligomeric compounds of the present invention were designed to target several human let-7-like genes. Additionally, a series of target-specific DNA oligonucleotide probes were synthesized by IDT (Coralville, IA) and used in Northern analyses to assess the expression of let-7-like miRNA homologs in various tissues. These let-7 homolog specific probes are shown in Table 22.

Table 22

Probes for Northern analyses of mRNA expression of let-7 homologs

ISIS Number	SEQ ID NO	Sequence	pri-miRNA
327890	308	AGCACAAACTACTACCTCA	let-7i
327893	311	AACCACACAACCTACTACCTCA	let-7b
327903	321	AACTATACAACCTACTACCTCA	let-7a-3
327926	344	ACTATGCAACCTACTACCTCT	let-7d
327930	348	ACTATACAACCTCCTACCTCA	let-7e
327931	349	AACCATACAACCTACTACCTCA	let-7c
327967	385	ACTGTACAAACTACTACCTCA	let-7g

For Northern analyses with let-7 homolog probes, total RNA from spleen, kidney, testes, heart, and liver tissues as well as total RNA from HEK293, T47D, T-24, MCF7, HepG2, and K-562 Leukemia cell lines was either prepared as described above or purchased from Ambion, Inc. (Austin, TX). Northern blotting was performed as described above (Example 14). The let-7c miRNA was observed to be expressed in spleen, kidney, testes, heart and liver tissues, as well as in HEK293 and T47D cell lines. The let-7e miRNA was observed to be expressed in T-24, MCF7, T47D, 293T, HepG2, and K-562 cell lines.

In one embodiment, expression of let-7-like pri-miRNA homologs was detected in total RNA from brain, liver and spleen tissues, as well as total RNA from preadipocytes, differentiated adipocytes, and HeLa, HEK-293, and T-24 cell lines by real-time RT-PCR. Primer/probe sets were designed to distinguish between and amplify specific let-7-like pri-miRNA homologs. These primer/probe sets are shown in Table 23.

Table 23

Primer/probe sets for assaying expression of let-7 miRNA homologs

Pri-miRNA	Primer or probe	Isis number	SEQ ID NO.	sequence
let-7b	forward	341672	765	GAGGTAGTAGGTTGTGTGGTTTCA
	reverse	341673	766	AGGGAAGGCAGTAGGTTGTATAGTT
	probe	341674	767	CAGTGATGTTGCCCCTCGGAAGA
let-7c	forward	341675	768	TGCATCCGGGTTGAGGTA
	reverse	341676	769	AGGAAAGCTAGAAGGTTGTACAGTTAA
	probe	341677	770	AGGTTGTATGGTTTAGAGTTACACCCTGGGA
let-7d	forward	341678	771	CCTAGGAAGAGGTAGTAGGTTGCA
	reverse	341679	772	CAGCAGGTCGTATAGTTACCTCCTT
	probe	341680	773	AGTTTTAGGGCAGGGATTTTGCCCA
let-7g	forward	341681	774	TTCCAGGCTGAGGTAGTAGTTTG

	reverse	341682	775	TTATCTCCTGTACCGGGTGGT
	probe	341683	776	ACAGTTTGAGGGTCTAT
let-7i	forward	341684	777	TGAGGTAGTAGTTTGTGCTGTTGGT
	reverse	341685	778	AGGCAGTAGCTTGCGCAGTTA
	probe	341686	779	TTGTGACATTGCCCGCTGTGGAG
let-7a-1	forward	341687	780	GGATGAGGTAGTAGGTTGTATAGTTTTAGG
	reverse	341688	781	CGTTAGGAAAGACAGTAGATTGTATAGTTATC
	probe	341689	782	TCACACCCACCACTGG
let-7a-3	forward	341690	783	GGGTGAGGTAGTAGGTTGTATAGTTTGG
	reverse	341691	784	CACTTCAGGAAAGACAGTAGATTGTATAGTT
	probe	341692	785	CTCTGCCCTGCTATGG

Using these primer/probe sets, the let-7-like pri-miRNA homologs were found to be expressed in human brain, liver and spleen, as well as preadipocytes, differentiated adipocytes, and HeLa, T-24 and HEK-293 cells lines. In particular, the let-7b pri-miRNA exhibited approximately 100-fold higher expression in differentiated adipocytes as compared to preadipocytes. Furthermore, the let-7b, let-7c, let-7d, let-7i, and let-7a-3 pri-miRNAs were highly expressed in brain and spleen tissues.

In summary, the let-7-like homologs have been found to be widely expressed in various human tissues and several cell lines. Furthermore, some oligomeric compounds targeted to human let-7 pri-miRNAs generally appeared to result in the induction of cell differentiation, consistent with the functional role of *let-7* as a regulator of developmental timing in nematode larva. Specifically, the oligomeric compounds targeted to let-7c (ISIS Number 327931; SEQ ID NO: 349) and let-7a-3 (ISIS Number 327903; SEQ ID NO: 321) resulted in an increase in expression levels for several markers of adipocyte differentiation. Furthermore, inhibition of the let-7-like homologs by oligomeric compounds of the present invention did not appear to induce caspases activated in apoptotic pathways (performed in Example 15). Thus, the oligomeric compounds of the present invention targeting let-7-like pri-miRNA homologs appear to stimulate adipocyte differentiation and do not promote cell death by apoptosis. Thus, the oligomeric compounds of the present invention may be useful as pharmaceutical agents in the treatment of anorexia or diseases, conditions or injuries in which the induction of cellular differentiation is desireable, such as Alzheimers disease or central nervous system injury, in which neural regeneration would be beneficial.

Example 18: Effects of oligomeric compounds targeting miRNAs on insulin signaling in HepG2 cells

Insulin is secreted from pancreatic β-cells in response to increasing blood glucose levels. Through the regulation of protein expression, localization and activity, insulin ultimately stimulates conversion of excess glucose to glycogen, and results in the restoration of blood

glucose levels. Insulin is known to regulate the expression of over 100 gene products in multiple cell types. For example, insulin completely inhibits the expression of hepatic insulin-like growth factor binding protein-1 (IGFBP-1), a protein which can sequester insulin-like growth factors, and phosphoenolpyruvate carboxykinase-cytosolic (PEPCK-c) which is a rate-controlling enzyme of hepatic gluconeogenesis. Levels of the follistatin mRNA are also believed to decrease in response to insulin treatment. IGFBP-1 and PEPCK-c are overexpressed in diabetes, and PEPCK-c overexpression in animals promotes hyperglycemia, impaired glucose tolerance and insulin-resistance. Thus, the IGFBP-1, PEPCK-c and follistatin genes serve as marker genes for which mRNA expression can be monitored and used as an indicator of an insulin-resistant state. Oligomeric compounds with the ability to reduce expression of IGFBP-1, PEPCK-c and follistatin are highly desirable as agents potentially useful in the treatment of diabetes and hypertension.

Oligomeric compounds of the present invention were tested for their effects on insulin signaling in HepG2 cells. HepG2 cells were plated at 7500 cells/well in collagen coated 96-well plates. The following day, cells were transfected with oligomeric compounds targeting miRNAs using 100nM oligomeric compound in LIPOFECTIN™ (Invitrogen Corporation, Carlsbad, CA) in two 96-well plates. The oligomeric compounds were tested in triplicate on each 96-well plate, except for positive and negative controls, which were measured up to six times per plate. At the end of transfection, the transfection medium was replaced by regular growth medium. Twenty-eight hours post-transfection, the cells were subjected to overnight (sixteen to eighteen hours) serum starvation using serum free growth medium. Forty-four hours post-transfection, the cells in the transfected wells were treated with either no insulin ("basal" Experiment 1, for identification of insulin-mimetic compounds) or with 1nM insulin ("insulin treated" Experiment 2, for identification of insulin sensitizers) for four hours. At the same time, in both plates, cells in some of the un-transfected control wells are treated with 100nM insulin to determine maximal insulin response. At the end of the insulin or no-insulin treatment (forty-eight hours post-transfection), total RNA is isolated from both the basal and insulin treated (1nM) 96-well plates, and the amount of total RNA from each sample is determined using a Ribogreen assay (Molecular Probes, Eugene, OR). Real-time PCR is performed on all the total RNA samples using primer/probe sets for three insulin responsive genes: PEPCK-c, IGFBP-1 and follistatin. Expression levels for each gene are normalized to total RNA, and values ± standard deviation are expressed relative to the transfectant only untreated control (UTC) and negative control compounds. Results of these experiments are shown in Tables 24 and 25.

Table 24

Experiment 1: Effects of oligomeric compounds targeting miRNAs on insulin-repressed gene expression in HepG2 cells

ISIS Number	SEQ ID NO	Pri-miRNA	IGFBP-1 (% UTC)	PEPCK-c (% UTC)	Follistatin (% UTC)
UTC	N/A	N/A	100	100	100
29848 n-mer	737	N/A	95	87	94
327876	294	mir-29b-1	93	119	104
327878	296	mir-203	162	45	124
327880	298	mir-10b	137	110	107
327889	307	mir-23b	56	137	56
327890	308	let-7I	99	85	78
327892	310	mir-131-2 /mir-9	108	75	91
327901	319	mir-143	133	119	93
327903	321	let-7a-3	71	71	60
327905	323	mir-205	107	129	104
327913	331	mir-29c	123	229	115
327919	337	mir-221	96	71	74
327922	340	mir-19b-2	109	77	57
327925	343	mir-133b	152	145	110
327933	351	mir-145	125	118	112
327934	352	mir-213	231	99	140
327939	357	mir-125b-1	125	125	104
327941	359	mir-181b	83	101	80
327954	372	mir-148b	118	79	100
338664	491	mir-143 pri-miRNA	90	75	93
340927	319	mir-143	201	87	111

Under "basal" conditions (without insulin), treatments of HepG2 cells with oligomeric compounds of the present invention resulting in decreased mRNA expression levels of the PEPCK-c, IGFBP-1 and/or follistatin marker genes indicate that the oligomeric compounds have an insulin mimetic effect. Treatments with oligomeric compounds of the present invention resulting in an increase in mRNA expression levels of the PEPCK-c, IGFBP-1 and/or follistatin marker genes indicate that these compounds inhibit or counteract the normal insulin repression of mRNA expression of these genes.

From these data, it is evident that the oligomeric compounds, ISIS Number 327878 targeting mir-203 and ISIS Number 327922 targeting mir-19b-2, for example, result in a 55% and a 23% decrease, respectively, in PEPCK-c mRNA, a marker widely considered to be insulin-responsive. Thus, these oligomeric compounds may be useful as pharmaceutic agents comprising insulin mimetic properties in the treatment, amelioration, or prevention of diabetes or other metabolic diseases.

Conversly, the results observed with the oligomeric compound targeting mir-29c (ISIS Number 327913), for example, exhibiting increased expression of the IGFBP-1, PEPCK-c and follistatin marker genes, suggest that the mir-29c miRNA target may be involved in the

regulation of these insulin-responsive genes. When the mir-29c miRNA is inactivated by an oligomeric compound, IGFBP-1, PEPCK-c and follistatin gene expression is no longer repressed.

Table 25

Experiment 2: Effects of oligomeric compounds targeting miRNAs on insulin-sensitization of gene expression in HepG2 cells

ISIS Number	SEQ ID NO	Pri-miRNA	IGFBP-1 (% UTC)	PEPCK-c (% UTC)	Follistatin (% UTC)
UTC(1 nm insulin)	N/A	N/A	100	100	100
29848 n-mer	737	N/A	92	94	97
327876	294	mir-29b-1	118	176	138
327878	296	mir-203	185	29	150
327880	298	mir-10b	136	125	149
327890	307	let-7i	88	113	115
327892	308	mir-131-2 /mir-9	139	104	96
327901	310	mir-143	135	117	135
327903	319	let-7a-3	81	87	89
327905	321	mir-205	115	147	148
327913	323	mir-29c	147	268	123
327919	331	mir-221	154	105	178
327922	337	mir-19b-2	104	76	61
327925	340	mir-133b	166	182	148
327933	343	mir-145	179	115	185
327934	351	mir-213	244	105	103
327939	352	mir-125b-1	175	153	192
327941	357	mir-181b	80	98	68
327954	359	mir-148b	120	102	105
327889	372	mir-23b	73	202	72
338664	491	mir-143 pri-miRNA	100	76	84
340927	319	mir-143	285	103	128

For HepG2 cells treated with 1nM insulin, treatments with oligomeric compounds of the present invention resulting in a decrease in mRNA expression levels of the PEPCK-c, IGFBP-1 and/or follistatin marker genes indicate that these compounds have an insulin sensitization effect. Treatments with oligomeric compounds of the present invention resulting in an increase in mRNA expression levels of the PEPCK-c, IGFBP-1 and/or follistatin marker genes indicate that these compounds inhibit or counteract the normal insulin response of repression of mRNA expression of these genes.

From these data, it is evident that the oligomeric compounds, ISIS Number 327878 targeting mir-203 and ISIS Number 327922 targeting mir-19b-2, for example, were observed to result in a 71% and a 24% reduction, respectively, of PEPCK-c mRNA expression, widely considered to be a marker of insulin-responsiveness. Thus, these oligomeric compounds may be useful as pharmaceutic agents with insulin-sensitizing properties in the treatment, amelioration,

or prevention of diabetes or other metabolic diseases.

Conversly, the results observed with the oligomeric compounds targeting mir-29c (ISIS Number 327913), mir-133b (ISIS Number 327925), and mir-125b-1 (ISIS Number 327939), all exhibiting increased expression of the IGFBP-1, PEPCK-c and follistatin marker genes, support the conclusion that the mir-29c, mir-133b, and mir-125b-1 miRNAs may be involved in the regulation of insulin-responsive genes. When these miRNAs are inactivated by the oligomeric compounds of the present invention, IGFBP-1, PEPCK-c and follistatin gene expression is no longer repressed or insulin-sensitive.

A caspase assay was also performed (as in Example 11 above) in HepG2 cells treated with oligomeric compounds of the present invention, and it was determined that oligomeric compounds targeting the mir-29c, mir-133b, and mir-125b-1 miRNAs were not toxic to the cells and that the observed reduction in mRNA expression levels of insulin-responsive genes was not due to a general toxicity of the compounds or an induction of apoptotic pathways.

Example 19: Analysis of expression of mir-143 pri-miRNA and mature mir-143

Ribonuclease protection assays:

The ribonuclease protection assay (RPA) is known in the art to be a sensitive and accurate method of measuring and/or following temporal changes in the expression of one or more RNA transcripts in a complex mixture of total RNA. Briefly, this method employs a radioactive probe that specifically hybridizes to a target transcript RNA. The probe is added to a sample of total RNA isolated from tissues or cells of interest, and, upon hybridization to its target, the probe forms a double-stranded RNA region. If the region of hybridization is shorter than the entire length of either the probe or the target RNA molecule, the molecule will be a hybrid molecule with partial double-stranded and partial single-stranded character. These hybrid molecules are then digested with single-strand-specific RNases such as RNase A and/or T1, which remove any non-hybridized single stranded portions of the hybrid molecules, leaving only the "protected" dsRNA fragments. The RNase protected fragments are then electrophoresed on a denaturing gel, causing the strands to dissociate, and the intensity of radioactive probe signal observed is directly proportional to the amount of specific target transcript RNA in the original total RNA sample.

In an embodiment of the present invention, small non-coding RNAs in a sample were detected by RPA using probes that hybridize to pri-miRNAs, pre-miRNAs or mature miRNAs. Probes were *in vitro* transcribed using the *mir*Vana™ miRNA Probe Construction Kit (Ambion Inc., Austin, TX) according to the manufacturer's protocol, beginning with a DNA

oligonucleotide representing sense strand of the mature miRNA to be detected plus four thymidylate residues plus an 8-base sequence complementary to the 3'-end of the T7 promoter primer supplied with the kit. When the T7 primer is annealed to this DNA oligonucleotide, the Klenow DNA polymerase is used to generate a double-stranded DNA, and then *in vitro* transcription is performed using the T7 RNA polymerase and radiolabeled nucleotides to generate a radioactive RNA probe for detection of the miRNA.

In one embodiment, a probe specifically hybridizing to the murine mir-143 miRNA was used in a RPA of 5 μg total RNA from kidney, liver, heart, lung, brain, spleen, and thymus tissues from mouse as well as adipose tissue from db/db obese mice, total RNA from an 11-day-old embryo, and total RNA from undifferentiated and differentiated 3T3-L1 cells. All signals were normalized to the levels of 5.8S rRNA. Expression levels of mir-143 were highest in lung, heart, spleen, thymus and kidney tissues from wildtype mice. Notably, mir-143 expression levels were significantly elevated in adipose tissue from db/db mice (approximately 4 times higher than expression levels in kidney, 2.4 times higher than levels in heart and 1.6 times higher than levels in lung tissues from wildtype mice).

In one embodiment, a probe hybridizing to the mir-143 pri-miRNA molecule was used in a RPA of 2-5 μg total RNA from human spleen, thymus, testes, heart, liver, kidney, skeletal muscle, brain, lung and adipose tissues, as well as total RNA from preadipocytes, differentiated adipocytes, and HepG2 cells. A probe hybridizing to the β-actin mRNA was used as a control. The highest levels of mir-143 pri-miRNA were observed in heart, kidney, thymus and adipose tissues, as well as in differentiated adipocytes.

In one embodiment, a probe hybridizing to the mature mir-143 miRNA was also used in a RPA of 2 μg total RNA from human spleen, thymus, heart, liver, kidney and brain, tissues, as well as total RNA from preadipocytes, differentiated adipocytes, and total RNA from HepG2, A549, T-24, HEK293, HuVEC (human umblical vein endothelial cells), HL-60 and T47D cell lines. A probe hybridizing to the β-actin mRNA was used as a control, and all signals were normalized to the levels of mir-143 expression in preadipocytes. The results are shown in Table 26.

Table 26

RNase protection of mature mir-143 in total RNA from tissues and cell lines

Tissue or cell line	Fold Increase over preadipocytes
Spleen	2.6
Thymus	3.8
Heart	8.2
Liver	0
Kidney	10.0

Brain	0.9
Preadipocytes	1.0
Differentiated adipocytes	2.6
HepG2	0.5
A549	N.D.
T-24	0.4
HEK293	0.5
HuVEC	0.3
HL-60	0.4
T47D	0.3

From these data, the highest levels of expression of the mature mir-143 miRNA were observed in total RNA from kidney and heart tissues. High levels of expression of the mature mir-143 miRNA were also observed in total RNA from lymphoid tissues such as spleen and thymus. Expression of the mature mir-143 miRNA is increased in differentiated adipocytes as compared to levels in preadipocytes. These data also suggest that the mir-143 miRNA plays a role in cellular differentiation.

In one embodiment, a uniform 2'-MOE phosphorothioate oligomeric compound with a sequence antisense to the mature mir-143 miRNA was spiked into the RPA mixture above. This antisense mir-143 compound was found to block the ribonuclease protection expression pattern previously observed, suggesting that this antisense mir-143 oligomeric compound specifically hybridizes to and inhibits the activity of mir-143. This oligomeric compound targeting the mir-143 miRNA is predicted to form a double stranded molecule that blocks endogenous mir-143 miRNA activity when employed *in vivo*.

It was also noted that, while expression of the mir-143 miRNA can be detected in non-transformed cells, such as HuVECs, in general, transformed cell lines have not been observed to exhibit high levels expression of mir-143. When taken together with the observation that the mir-143 miRNA is upregulated as adipocytes differentiate as well as the observation that oligomeric compounds targeting mir-143 inhibit adipocyte differentiation, these data suggest that mir-143 normally promotes adipocyte differentiation and mir-143 may have an inhibitory effect on cellular transformation that is consistent with its role in promoting cellular differentiation. Lack of expression or downregulation of mir-143 in transformed cell lines may be a cause or consequence of the undifferentiated state. Thus, mir-143 mimics may be useful as pharmaceutical agents in the treatment of hyperproliferative disorders such as cancer.

In one embodiment, the expression of human mir-143 was assessed during adipocyte differentiation. A probe hybridizing to the human mir-143 miRNA was used in a RPA of 5 μg total RNA from pre-adipocytes, and differentiated adipocytes sampled at one, four, and ten days post-differentiation. All signals were normalized to the levels of 5.8S rRNA. mir-143 expression

levels were 2.5 to 3-fold higher by day 10 post-differentiation when compared to mir-143 expression levels in pre-adipocytes by ribonuclease protection assay.

Real-time RT-PCR analysis of mir-143 pri-miRNA expression:

Expression levels of mir-143 pri-miRNA were compared in total RNAs from various tissues and total RNA from several cell lines. Total RNA from spleen, heart, liver, and brain tissues, as well as total RNA from preadipocytes, differentiated adipocytes, and HepG2, T-24 and HeLa cell lines was purchased or prepared as described *supra*. 80 ng of total RNA from each source was used to perform real-time RT-PCR using a primer/probe set specific for the mir-143 pri-miRNA molecule. ISIS 339314 (TCCCAGCCTGAGGTGCA; SEQ ID NO: 786) was used as the forward primer, ISIS 342897 (GCTTCATCTCAGACTCCCAACTG; SEQ ID NO: 787) was used as the reverse primer, and ISIS 342898 (TGCTGCATCTCTG; SEQ ID NO: 788) was used as the probe. RNA levels from all sources were compared to RNA levels from preadipocytes. Greater than 32-fold higher levels of mir-143 pri-miRNA were observed in heart tissue as compared to preadipocytes; 19-fold higher levels of mir-143 pri-miRNA were observed in differentiated adipocytes relative to levels in preadipocytes; 5-fold higher levels of mir-143 pri-miRNA were observed in spleen as compared to preadipocytes.

Northern blot analyses were performed in differentiating adipocytes as described in Example 14 using the mir-143-specific DNA oligonucleotide probe (SEQ ID NO: 319) to detect the mir-143 target and a probe for the U6 RNA to normalize for variations in loading and transfer efficiency, and it was confirmed by Northern analysis that expression of mature mir-143 increases from day 1 through day 10 after induction of differentiation.

In human pre-adipocytes and adipocytes sampled one, four, seven and ten days post-differentiation, expression levels of mir-143 pri-miRNA were also assessed using real-time RT-PCR analysis as described herein. 80 ng of total RNA from pre-adipocytes or differentiated adipocytes was used to perform real-time RT-PCR using the same primer/probe set specific for the mir-143 pri-miRNA molecule described supra (ISIS 339314, SEQ ID NO: 786 was used as the forward primer, ISIS 342897, SEQ ID NO: 787 was used as the reverse primer, and ISIS 342898, SEQ ID NO: 788 was used as the probe). RNA levels from all sources were normalized to 5.8S rRNA levels. mir-143 pri-miRNA levels in preadipocytes were 94% of the level of the 5.8S rRNA. At day 1 post-differentiation, mir-143 pri-miRNA levels had decreased to 38% of the level of the 5.8S rRNA. By day 4 post-differentiation, mir-143 pri-miRNA levels had decreased to 26%, by day 7 post-differentiation, mir-143 pri-miRNA levels were at 25%, and by day 10 post-differentiation, mir-143 pri-miRNA levels had dropped to 23% of the level of the 5.8S rRNA. Taken together with the results from RPA analysis, it appears that levels of the

mature mir-143 miRNA increases approximately 2- to 3-fold by day 10 post-differentiation in differentiated adipocytes, accompanied by a concomittant approximately 4-fold decrease in the levels of unprocessed mir-143 pri-miRNA, indicating that adipocyte differentiation coincides with either an increase in processing of the mir-143 miRNA from the mir-143 pri-miRNA or an overall decrease in mir-143 pri-miRNA production.

Effects of oligomeric compounds on expression of pri-miRNAs:

Mature miRNAs originate from long endogenous primary transcripts (pri-miRNAs) that are often hundreds of nucleotides in length. It is believed that a nuclear enzyme in the RNase III family, known as Drosha, processes pri-miRNAs (which can range in size from about 110 nucleotides up to about 450 nucleotides in length) into pre-miRNAs (from about 70 to 110 nucleotides in length) which are subsequently exported from the nucleus to the cytoplasm, where the pre-miRNAs are processed by human Dicer into double-stranded intermediates resembling siRNAs, which are then processed into mature miRNAs. Using the real-time RT-PCR methods described herein, the expression levels of several pri-miRNAs were compared in differentiating adipocytes. Total RNA from preadipocytes and differentiating adipocytes was prepared as described herein.

In one embodiment, modified oligomeric compounds can be transfected into preadipocytes or other undifferentiated cells, which are then induced to differentiate (as described in detail, herein), and it can be determined whether these modified oligomeric compounds act to inhibit or promote cellular differentiation. Real-time RT-PCR methods can then be used to determine whether modified oligomeric compounds targeting miRNAs can affect the expression or processing of the pre-miRNAs from the pri-miRNA (by the Drosha enzyme), the processing of the mature miRNAs from the pre-miRNA molecules (by the Dicer enzyme), or the RISC-mediated binding of a miRNA to its target nucleic acid.

Here, oligomeric compounds targeting mir-143 were transfected into preadipocytes which were then induced to differentiate, in order to assess the effects of these compounds on mir-143 pri-miRNA levels during differentiation. mir-143 pri-miRNA levels were assessed on days 3 and 9 after differentiation.

In addition to the uniform 2'-MOE phosphorothioate oligomeric compound ISIS Number 327901 (SEQ ID NO: 319) targeting mature mir-143, a 5-10-7 gapmer oligomeric compound, ISIS Number 340927 (SEQ ID NO: 319), was designed to target mature mir-143. As negative controls, "scrambled" oligomeric compounds were also designed; ISIS Number 342672 (ATACCGCGATCAGTGCATCTTT; incorporated herein as SEQ ID NO: 789) contains 13 mismatches with respect to the mature mir-143 miRNA, and ISIS Number 342673 (SEQ ID NO:

758) contains 15 mismatches with respect to the mature mir-143 miRNA. ISIS 342672 and ISIS 342673 are uniform 2'-MOE phosphorothioate oligomeric compounds 22 nucleotides in length. ISIS Number 342677 (SEQ ID NO: 789) and ISIS Number 342678 (SEQ ID NO: 758) are the corresponding 5-10-7 scrambled 2'-MOE gapmer oligomeric compounds. All cytidine residues are 5-methylcytidines. Additionally, ISIS Number 342683 (CCTTCCCTGAAGGTTCCTCCTT; herein incorporated as SEQ ID NO: 790), representing the scrambled sequence of an unrelated PTP1B antisense oligonucleotide, was also used as a negative control.

These compounds were transfected into differentiating adipocytes and their effects on levels of the mir-143 pri-miRNA molecule were assessed in pre-adipocytes vs. differentiated adipocytes, by real-time RT-PCR using the primer/probe set specific for the mir-143 pri-miRNA (forward primer=ISIS 339314, SEQ ID NO: 786; reverse primer=ISIS 342897, SEQ ID NO.: 787; probe=ISIS 342898, SEQ ID NO.: 788). Thus, it was observed that in the presence of the oligomeric compound ISIS Number 327901 (SEQ ID NO: 319), levels of the mir-143 pri-miRNA are enhanced approximately 4-fold in differentiated adipocytes 9 days post-differentiation as compared to 3 days post-differentiation. These results suggest that ISIS Number 327901, the uniform 2'-MOE P=S oligomeric compound targeted to mature mir-143, interferes with the processing of the mir-143 pri-miRNA into the pre-miRNA by the Drosha RNase III enzyme. Alternatively, the compound interferes with the processing of the mir-143 pre-miRNA into the mature mir-143 miRNA by the Dicer enzyme. The decrease in levels of mature mir-143 miRNA in differentiating cells treated with ISIS Number 327901 (SEQ ID NO: 319) may also trigger a feedback mechanism that signals these cells to increase production of the mir-143 pri-miRNA molecule. Not mutually exclusive with the processing interference or the feedback mechanisms is the possibility that treatment with oligomeric compounds could stimulate the activity of an RNA-dependent RNA polymerase (RdRP) that amplifies the mir-143 pri-miRNA or pre-miRNA molecules. Oligomeric compounds of the present invention are predicted to disrupt pri-miRNA and/or pre-miRNA structures, and sterically hinder Drosha and/or Dicer cleavage, respectively. Furthermore, oligomeric compounds which are capable of binding to the mature miRNA are also predicted to prevent the RISC-mediated binding of a miRNA to its target nucleic acid, either by cleavage or steric occlusion of the miRNA.

Example 20: Identification of RNA transcripts bound by miRNAs

The RACE-PCR method (Rapid Amplification of cDNA Ends) was used as a means of identifying candidate RNA transcripts bound and/or potentially regulated by miRNAs. RNA was prepared and isolated from preadipocytes, and, using the SMART RACE cDNA

Amplification kit (BD Biosciences, Clontech, Palo Alto, CA) according to manufacturer's protocol, synthetic adaptor sequences were incorporated into both the 5'- and 3'-ends of the amplified cDNAs during first strand cDNA synthesis. 5' RACE-PCR was then performed using the mature miRNA as the 3'-end primer along with the 5' adapter primer from the kit to amplify the 5'-end of the candidate RNA transcript. 3' RACE-PCR was performed using the antisense sequence of the miRNA as a primer along with the 3' adapter primer from the kit to amplify the 3'-end of the candidate RNA transcript. In some embodiments, the primers 2-nucleotides shorter than the corresponding miRNA were used in order to identify targets with some mismatching nucleotides at the end of the miRNA (these primers are indicated by "3'-RACE-2nt" in Table 27 below).

For example, the antisense sequences of the mature mir-43, let-7g, mir-23b, mir-29c, mir-131, mir-143, mir-130b and mir-213 miRNAs were used as primers in 3' RACE-PCR, and the mature mir-143 or mir-15a sequences were used in 5' RACE-PCR. The RACE-PCR products employing the mir-143 miRNA, the mir-143 antisense sequence, the mir-131 antisense sequence or the mir-15a miRNA as primers were electrophoresed and gel purified, prominent bands were excised from the gel, and these products were subcloned using standard laboratory methods. The subcloned products from the RACE-PCR were then were sent to Retrogen, Inc. (San Diego, CA) for sequencing. Candidate RNA transcripts targeted by miRNAs were thereby identified.

Candidate RNA targets identified by RACE-PCR methods are shown in Table 27, where the miRNA-specific primer used to identify each transcript is indicated in the column entitled "primer". (In some cases, the target was identified multiple times by more than one RACE-PCR method, and thus appears in the table more than once).

Table 27

Predicted RNA targets of mir-143

Primer	Method	GenBank Accession	RNA transcript targeted by miRNA	SEQ ID NO
mir-143	5'RACE	NM_001753.2	caveolin 1, caveolae protein, 22kDa	791
mir-143	5'RACE	NM_004652.1	ubiquitin specific protease 9, X-linked (fat facets-like, Drosophila)	792
mir-143	5'RACE	NM_007126.2	valosin-containing protein	793
mir-143	5'RACE	NM_000031.1	aminolevulinate, delta-, dehydratase	794
mir-143	5'RACE	NM_007158.1	NRAS-related gene	795
mir-143	5'RACE	NM_015396.1	HSPC056 protein	796
mir-143	5'RACE	NM_001219.2	calumenin	797
mir-143	5'RACE	BC051889.1	RNA binding motif, single stranded interacting protein 1	798
mir-143	5'RACE	BX647603.1	Homo sapiens mRNA; cDNA DKFZp686L01105 (from clone DKFZp686L01105)	799

mir-143	5'RACE	AB051447.1	KIAA1660 protein	800
mir-143	5'RACE	NM_007222.1	zinc-fingers and homeoboxes 1	801
mir-143	5'RACE	NM_001855.1	collagen, type XV, alpha 1	802
mir-143	3'RACE	NM_007222.1	zinc-fingers and homeoboxes 1	801
mir-143	3'RACE	NM_006732	FBJ murine osteosarcoma viral oncogene homolog B	803
mir-143	3'RACE	NM_003718.2	cell division cycle 2-like 5 (cholinesterase-related cell division controller)	804
mir-143	3'RACE	NM_005626.3	splicing factor, arginine/serine-rich 4	805
mir-143	3'RACE	NM_002355.1	mannose-6-phosphate receptor (cation dependent)	806
mir-143	3'RACE	NM_000100.1	cystatin B (stefin B)	807
mir-143	3'RACE	NM_015959.1	CGI-31 protein	808
mir-143	3'RACE	NM_006769.2	LIM domain only 4	809
mir-143	3'RACE	NM_003184.1	TAF2 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 150kDa	810
mir-143	3'RACE	NM_025107.1	myc target in myeloid cells 1	811
mir-143	3'RACE	NM_003113.1	nuclear antigen Sp100	812
mir-143	3'RACE	NM_002696.1	polymerase (RNA) II (DNA directed) polypeptide G	813
mir-143	3'RACE	NM_004156.1	protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform	814
mir-143	3'RACE	NM_031157	heterogeneous nuclear ribonucleoprotein A1	815
mir-143	3'RACE	NM_004999.1	myosin VI	817
mir-143	3'RACE	NM_018036.1	chromosome 14 open reading frame 103	818
mir-143	3'RACE	NM_018312.2	chromosome 11 open reading frame 23	819
mir-143	3'RACE	NM_002950.1	ribophorin I	820
mir-143	3'RACE	NM_006708.1	glyoxalase I	821
mir-143	3'RACE	NM_014953.1	mitotic control protein dis3 homolog	822
mir-143	3'RACE	NM_004926.1	zinc finger protein 36, C3H type-like 1	823
mir-143	3'RACE	NM_004530.1	matrix metalloproteinase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)	824
mir-143	3'RACE	NM_015208.1	KIAA0874 protein	825
mir-143	3'RACE	NM_002582.1	poly(A)-specific ribonuclease (deadenylation nuclease)	826
mir-143	3'RACE	NM_000297.2	polycystic kidney disease 2 (autosomal dominant)	827
mir-143	3'RACE	NM_001175	Rho GDP dissociation inhibitor (GDI) beta	828
mir-143	3'RACE	XM_166529	glucocorticoid induced transcript 1, GLCCI1	837
mir-143	3'RACE -2nt	NM_001753.2	caveolin 1, caveolae protein, 22kDa	791
mir-143	3'RACE -2nt	NM_006732	FBJ murine osteosarcoma viral oncogene homolog B	803
mir-143	3'RACE -2nt	NM_000100.1	cystatin B (stefin B)	807
mir-143	3'RACE -2nt	NM_015959.1	CGI-31 protein	808
mir-143	3'RACE -2nt	NM_004156.1	protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform	814
mir-143	3'RACE	NM_031157	heterogeneous nuclear	815

	-2nt		ribonucleoprotein A1	
mir-143	3'RACE -2nt	NM_002582.1	poly(A)-specific ribonuclease (deadenylation nuclease)	826
mir-143	3'RACE -2nt	NM_000297.2	polycystic kidney disease 2 (autosomal dominant)	827
mir-143	3'RACE -2nt	NM_006325.2	RAN, member RAS oncogene family	829
mir-143	3'RACE -2nt	NM_004627.1	tryptophan rich basic protein	830
mir-143	3'RACE -2nt	NM_012210.1	tripartite motif-containing 32	831
mir-143	3'RACE -2nt	AJ131244.1	SEC24 related gene family, member A (S. cerevisiae)	832
mir-143	3'RACE -2nt	NM_031267.1	cell division cycle 2-like 5 (cholinesterase-related cell division controller)	833
mir-143	3'RACE -2nt	AL049367.1	guanine nucleotide binding protein (G protein), gamma 12	835
mir-143	3'RACE -2nt	NM_001344	defender against cell death 1	836
mir-131	3'RACE	AK001214.1	hypothetical protein FLJ10352	1735
mir-131	3'RACE	NM_001614	actin, gamma 1 (ACTG1), mRNA	1736
mir-131	3'RACE	NM_001948.1	dUTP pyrophosphatase (DUT), mRNA	1737
mir-131	3'RACE	NM_002387.1	mutated in colorectal cancers (MCC), mRNA	1738
mir-131	3'RACE	NM_004109.1	ferredoxin 1 (FDX1), nuclear gene encoding mitochondrial protein, mRNA	1739
mir-131	3'RACE	NM_004342.4	caldesmon 1 (CALD1), transcript variant 2, mRNA	1740
mir-131	3'RACE	NM_005572.2	lamin A/C (LMNA), transcript variant 2, mRNA	1741
mir-131	3'RACE	NM_015640.1	PAI-1 mRNA-binding protein (PAI-RBP1), mRNA	1742
mir-131	3'RACE	NM_017789.1	semaphorin 4C (SEMA4C), mRNA	1743
mir-131	3'RACE	NM_144697.1	hypothetical protein BC017397 (LOC148523), mRNA	1744
mir-131	3'RACE	NM_173710	NADH dehydrogenase 3 (MTND3), mRNA	1745
mir-15a	5'RACE	AF220018.1	Homo sapiens tripartite motif protein (TRIM2) mRNA	1746
mir-15a	5'RACE	M98399.1	Human antigen CD36 mRNA	1747
mir-15a	5'RACE	Y00281.1	Human mRNA for ribophorin I	1748

Because these RNA transcripts in Table 27 were identified as being bound by one of the mir-143, mir-131, or mir-15a miRNAs, these miRNAs are predicted to serve a regulatory role in expression or activity of these transcripts identified by RACE-PCR. Additional candidate human RNA targets can be identified in the same manner.

Example 21: Effects of oligomeric compounds on adipocyte differentiation hallmark genes in differentiated adipocytes

The effect of the oligomeric compounds of the present invention targeting miRNAs on

the expression of markers of cellular differentiation was examined in differentiated adipocytes.

The effects of the oligomeric compounds of the present invention on the hallmark genes known to be upregulated during adipocyte differentiation assayed in Example 13 were also assayed in differentiated adipoctyes. As previously described, the HSL, aP2, Glut4, and PPARγ genes play important rolls in the uptake of glucose and the metabolism and utilization of fats. Also as previously described, an increase in triglyceride content is another well-established marker for adipocyte differentiation. Human white preadipocytes (Zen-Bio Inc., Research Triangle Park, NC) were grown in preadipocyte media (ZenBio Inc.). After the cells reached confluence (approximately three days), they were exposed to differentiation media (Zen-Bio, Inc.) containing a PPAR-γ agonist, IBMX, dexamethasone, and insulin for three days. Cells were then fed Adipocyte Medium (Zen-Bio, Inc.), which was replaced at 2 to 3 day intervals. One day before transfection, 96-well plates were seeded with 3000 cells/well. Cells were then transfected on day nine post-differentiation, according to standard published procedures with 250nM oligonucleotide in LIPOFECTIN™ (Invitrogen Corporation, Carlsbad, CA) (Monia et al., *J. Biol. Chem.* **1993** *268*(19):14514-22). Oligomeric compounds were tested in triplicate on each 96-well plate, and the effect of TNF-α, known to inhibit adipocyte differentiation, was also measured in triplicate. Oligomeric compound treatments and transfectant-only negative controls may be measured up to six times per plate. On day twelve post-differentiation, cells were washed and lysed at room temperature, and the expression of the four hallmark genes, HSL, aP2, Glut4, and PPARγ, as well as triglyceride (TG) accumulation were measured in adipocytes transfected with the uniform 2'-MOE phosphorothioate (PS) previously described in Example 13 as well as the chimeric gapmer oligomeric compounds targeting the mir-143 miRNA and the mir-143 pri-miRNA described in Example 16. On day twelve post-differentiation, cells were lysed in a guanadinium-containing buffer and total RNA was harvested. The amount of total RNA in each sample was determined using a Ribogreen Assay (Molecular Probes, Eugene, OR). Real-time PCR was performed on the total RNA using primer/probe sets for the adipocyte differentiation hallmark genes Glut4, HSL, aP2, and PPARγ. Triglyceride levels as well as mRNA levels for each of the four adipocyte differentiation hallmark genes are expressed relative to control levels (control = treatment with ISIS-29848 (SEQ ID NO: 737)). The results of this experiment are shown in Table 28.

Table 28

Effects of oligomeric compounds targeting miRNAs on expression of adipocyte differentiation markers

ISIS Number	SEQ ID NO	TG	aP2	HSL	Glut4	PPAR gamma
327876	294	1.16	0.67	0.81	3.53	1.28
327878	296	1.08	0.13	0.19	0.17	0.85
327880	298	1.12	1.14	0.93	0.76	1.86
327888	306	1.13	0.73	0.84	0.56	1.69
327889	307	1.09	1.12	0.77	0.99	1.63
327890	308	1.13	0.35	0.42	0.37	1.05
327892	310	1.23	0.81	0.62	0.42	1.01
327901	319	1.12	1.28	1.47	2.20	1.34
327903	321	1.12	0.56	0.53	0.36	0.91
327905	323	1.18	0.85	0.65	0.58	1.31
327913	331	1.12	1.05	1.09	1.52	1.29
327919	337	1.15	1.20	0.83	1.82	1.80
327922	340	1.48	0.91	1.01	0.61	0.99
327925	343	1.33	0.78	1.20	0.74	1.30
327933	351	1.63	1.58	1.30	2.12	1.60
327934	352	1.43	1.50	1.97	1.52	1.54
327939	357	1.33	1.16	1.08	0.72	1.89
327941	359	1.33	0.90	1.17	0.90	1.66
327954	372	1.46	1.23	1.35	0.61	1.46
328382	491	1.33	0.92	0.53	0.75	0.97
338664	491	1.72	0.77	1.01	1.08	1.06
340927	319	1.61	0.71	0.64	0.96	1.21

From these data, it was observed that the compound targeting the mir-203 miRNA (ISIS Number 327878), exhibited a sustained reduction in the hallmark marker genes at the 12th day post differentiation. Treatment with this compound resulted in decreased expression of the aP2, HSL, Glut4 and PPARγ marker genes, indicating that this oligomeric compound may lead to reduced levels of mobilization of fatty acids from adipose tissue, and has the potential to ameliorate some of the symptoms of type 2 diabetes, obesity, hypertension, atherosclerosis, cardiovascular disease, insulin resistance, and certain cancers. Notably, the effect of treatment of differentiated adipocytes with this oligomeric compound targeting the mir-203 miRNA mirrors the effect of treating cells with the TNF-α positive control that inhibits adipocyte differentiation. This evidence suggests that the oligomeric compound targeting the mir-203 miRNA can act as a TNF-α mimetic compound, and potentially may be used in the suppression of cellular differentiation and the maintenance of cells in a quiescent state.

The oligomeric compound targeting the mir-203 miRNA was also tested in the insulin assay (see Example 18) and was observed to reduce expression of PEPCK-c, indicating that it may also be useful as an insulin mimetic and/or antidiabetic drug.

As an extension of these conclusions, one having ordinary skill in the art would appreciate that further modified oligomeric compounds could be designed to also target the mir-203 mature miRNA, or the pri-miRNA and pre-miRNA precursors. Such compounds are noted to be within the scope of the present invention.

Example 22: Effects of oligomeric compounds on lymphocytic leukemia cells

Mir15-a-1 and mir-16-3 have been recently shown to reside in human chromosomal region (13q14) that is deleted in about 50% of chronic lymphocytic leukemia (CLL) patients. Mir-15 and 16 were found to be down-regulated in about 68% of CLL cases (Calin et al., Proc. Natl. Acad. Sci. USA, 2002, 99, 15524-15529, which is incorporated herein by reference in its entirety). CLL B-cells develop chemotherapy resistance over time, possibly due to a defective apoptosis pathway.

Using the 5'RACE method (described in Example 20), the CD36 mRNA was identified as one target regulated by mir-15 and/or mir-16 miRNAs. CD36 is a scavenger receptor involved in fat uptake by macrophages and adipocytes. CD36 is reported to be upregulated in some CLL cell lines, and its expression may correlate with tumor invasiveness.

If the apoptosis pathway is defective and the deletion or down-regulation of mir-15 and/or mir-16 play a role in CLL chemo-resistance, then addition of mir-15 and/or mir-16 should be able to induce apoptosis in CLL and increase drug-induced apoptosis. RNA oligonucleotide molecules ISIS Number 338963 (TAGCAGCACATAATGGTTTGTG; SEQ ID NO: 269) representing mir-15a-1/mir-15a-2, ISIS Number 338961 (TAGCAGCACATCATGGTTTACA; SEQ ID NO: 246) representing mir-15b, and ISIS Number 338965 (TAGCAGCACGTAAATATTGGCG; SEQ ID NO: 196) representing mir-16-1/mir-16-2/mir-16-3 were synthesized and deprotected. Additionally, RNA oligonucleotides bearing imperfect complementarity to these miRNA mimics (mimicking the imperfect complementarity found in the pri-miRNA) were also synthesized and deprotected. These imperfect complements were ISIS Number 338964 (TGCAGGCCATATTGTGCTGCCT; SEQ ID NO: 840), which is partially complementary to ISIS Number 338963 and represents the imperfect complement of mir-15a-1/mir-15a-2; ISIS Number 338962 (TGCGAATCATTATTTGCTGCTC; SEQ ID NO: 841), which is partially complementary to ISIS Number 338961 and represents the imperfect complement of mir-15b; ISIS Number 338966 (CTCCAGTATTAACTGTGCTGCTG; SEQ ID NO: 842), which is partially complementary to ISIS Number 338965 and represents the imperfect complement of mir-16-1 and mir-16-2; and ISIS Number 338967 (CACCAATATTACTGTGCTGCTT; SEQ ID NO: 843), which is partially complementary to ISIS Number 338965 and represents the imperfect complement of mir-16-3. These RNA molecules were diluted in water, and the concentration determined by A_{260}. Equimolar amounts of each of the miRNAs and their imperfect complementary RNA sequences were mixed together in the presence of Dharmacon 5X Universal buffer to form four "natural" double-stranded miRNA mimics. ISIS Number 338965 (SEQ ID NO: 196) was used twice; once, it was

hybridized to ISIS Number 338966, and once it was hybridized to ISIS Number 338967, to form two different "natural" double-stranded miRNA mimics, Mir-16-1/Mir-16-2 and Mir-16-3, with imperfect complementarity. The mixture of four "natural" miRNA mimics was incubated for 1-5 minutes at 90°C (the time depends on the volume of the mixture) and then incubated at 37°C for one hour. A_{260} readings were taken on the mixture for final concentration determination.

Heparinized peripheral blood from CLL patients was separated on a Ficoll density gradient to obtain greater than 95% pure CLL B-cells. These cells are tested for expression of the CD5/CD19/CD23 antigens. Positive expression of these three antigens indicates that the cells are CLL B-cells (Pederson et al., Blood, 2002, 100, 2965, which is incorporated herein by reference in its entirety). Additionally, cytogenetic analysis can be performed to ascertain that the cells have the 13q deletion. A mixture of all four "natural" miRNA mimics at 2 μM each was electroporated into the cells. The cells were cultured in the presence or absence of apoptosis-inducing agents fludarabine A, or Dexamethasone (which are known to employ the intrinsic mitochondrial apoptotic pathway) or the antitumor agent CDDO-Im (reported to function through an alternative extrinsic apoptotic pathway) for 24 hours. Following incubation, apoptosis was monitored by annexin/PI double staining as outlined in Figure 1 of Pederson et al., *Blood*, **2002**, *100*, 2965. The double-stranded RNA oligomeric compounds representing mir-15 and mir-16 miRNA mimics were observed to play a role in the induction of spontaneous as well as drug-induced apoptosis. Thus, oligomeric compounds of the present invention may be useful in the treatment of CD36-related diseases and conditions such as chronic lymphocytic leukemia and other cancers.

Example 23: Effect of oligomeric compounds targeting miRNAs *in vivo*

As described herein, leptin-deficient (ob/ob) mice, leptin receptor-deficient (db/db) mice and diet-induced obesity (DIO) mice are used to model obesity and diabetes. In accordance with the present invention, oligomeric compounds targeting mir-143, mir-131 (also known as mir-9) and mir-203 were tested in the ob/ob and db/db models. The ob/ob mice were fed a high fat diet and were subcutaneously injected with the oligomeric compounds of the invention or a control compound at a dose of 25 mg/kg two times per week for 6 weeks. Saline-injected animals, leptin wildtype littermates (i.e. lean littermates) and ob/ob mice fed a standard rodent diet served as controls. The physiological effects resulting from inhibition of target RNA, such as the effects of target inhibition on glucose and insulin metabolism and the expression of genes that participate in lipid metabolism, cholesterol biosynthesis, fatty acid oxidation, fatty acid storage, gluconeogenesis and glucose metabolism, were assessed by methods disclosed herein. In

brief, plasma levels of liver transaminases, cholesterol, triglycerides, free fatty acids and glucose were assessed weekly by tail bleed, with the tail bleed on week three taken under fasting conditions. After the treatment period, mice were sacrificed and liver, spleen, pancreas, muscle, kidney and heart, as well as brown adipose tissue (BAT) and white adipose tissue (WAT) tissues were collected. mRNA expression levels of the Glut4, aP2, HSL and PPARγ marker genes were evaluated. RNA isolation and target RNA expression level quantitation are performed as described.

Two oligomeric compounds targeting the mir-143 miRNA were compared for their effects on the physiological indications of obesity and diabetes. The oligomeric compound, ISIS Number 327901 (SEQ ID NO: 319), 22-nucleotides in length, targets the mature mir-143, and is a uniform 2'-MOE oligonucleotide with phosphorothioate internucleoside linkages throughout. The oligomeric compound ISIS Number 340927 (SEQ ID NO: 319) is a 5-10-7 gapmer also designed to target the mature mir-143 miRNA. The effects of these oligomeric compounds targeting mir-143 on several physiological parameters and markers of obesity and/or diabetes were examined *in vivo*. Potential effects on food consumption were also monitored.

Plasma cholesterol levels were observed to slightly decrease over time in ob/ob mice treated with the gapmer oligomeric compound ISIS Number 340927 (SEQ ID NO: 319) targeted to mir-143. Similarly, plasma triglyceride and plasma glucose levels were generally slightly lower in ob/ob mice treated with this compound as compared to untreated mice, or mice treated with control compounds. mRNA expression levels of the Glut4, aP2 and HSL marker genes were slightly reduced by both oligomeric compounds ISIS Number 327901 and ISIS Number 340927 targeting mir-143. Thus, these oligomeric compounds targeting mir-143 may be useful compounds in the treatment of obesity or diabetes.

In addition, Northern blot analyses were performed to quantitate the expression of mature mir-143 in kidney samples of ob/ob mice treated with oligomeric compounds of the present invention. The mir-143 specific DNA oligonucleotide probe (SEQ ID NO: 319) described above was used to detect expression levels of the mir-143 miRNA in ob/ob mice treated (twice weekly at 25 mg/kg) with ISIS Numbers 327901, the uniform 2'-MOE oligomeric compound, or ISIS Number 340927, the 5-10-7 gapmer compound, both targeted to mir-143, versus saline treated animals or animals treated with ISIS 342672 (SEQ ID NO: 789), a uniform 2'-MOE scrambled negative control oligomeric compound. Expression levels were normalized against the U6 RNA and the expression levels of saline treated animals were set at 100%. Most notably, in kidney samples from ob/ob mice treated with ISIS Number 327901, the uniform 2'-MOE oligomeric compound targeted to mir-143 exhibited a nearly 40% decrease in *in vivo*

expression levels of the mature mir-143 miRNA. In kidney samples from mice treated with the gapmer oligomeric compound targeting mir-143, ISIS Number 340927, a 23% reduction in *in vivo* expression levels of the mature mir-143 miRNA was observed.

Oligomeric compounds targeting the mir-131/mir-9 and the mir-203 miRNAs were also tested for their effects on the physiological indicators or markers of obesity and diabetes. The oligomeric compound, ISIS Number 327892 (SEQ ID NO: 310), targeted to mir-131/mir-9, 21-nucleotides in length, is a uniform 2'-MOE oligonucleotide with phosphorothioate internucleoside linkages throughout. The oligomeric compound ISIS Number 340926 (SEQ ID NO: 310) is a 5-10-6 gapmer oligomeric compound also designed to target the mir-131/mir-9 miRNA. The oligomeric compound ISIS Number 327878 (SEQ ID NO: 296) targeted to mir-203, 22-nucleotides in length, is a uniform 2'-MOE oligonucleotide with phosphorothioate internucleoside linkages throughout. The oligomeric compound ISIS Number 345349 (SEQ ID NO: 296) is a 5-10-7 gapmer oligomeric compound also designed to target the mir-203 miRNA. The effects of these oligomeric compounds were examined *in vivo* in the ob/ob model. Potential effects on food consumption were also monitored.

Fed plasma glucose levels in ob/ob mice treated with the oligomeric compounds ISIS Number 327892 (SEQ ID NO: 310) and ISIS Number 340926 (SEQ ID NO: 310) targeted to mir-131/mir-9, and ISIS Number 327878 (SEQ ID NO: 296) and ISIS Number 345349 (SEQ ID NO: 296) targeted to mir-203 were observed to be reduced beginning at approximately four weeks after the start of treatment and continuing to decrease on week five as compared to untreated mice, or mice treated with control compounds. Triglyceride levels were also observed to be reduced over time in mice treated with ISIS 340926 and 345349, the gapmer oligomeric compounds targeted to mir-131/mir-9 and mir-203, respectively. No signs of liver toxicity were indicated by weekly measurements of plasma transaminases upon treatment of ob/ob mice with any of the oligomeric compounds targeting mir-143, mir-203 or mir-131/mir9.

ob/ob mice in the fasted state on day 19 after treatment with the oligomeric compounds ISIS Number 327892 (SEQ ID NO: 310) and ISIS Number 340926 (SEQ ID NO: 310) targeted to mir-131/mir-9 also exhibited significant reductions in plasma glucose levels. Notably, the gapmer oligomeric compound ISIS Number 340926 (SEQ ID NO: 310) targeted to mir-131/mir-9 was even more potent than the corresponding uniform 2'-MOE oligonucleotide ISIS Number 327892 (SEQ ID NO: 310).

Furthermore, a decrease in food consumption was observed by the third week and this reduced level was maintained in the fourth week post-treatment of ob/ob mice with these oligomeric compounds. Therefore, the oligomeric compounds targeting the mir-131/mir-9 and

mir-203 miRNAs have potential use as appetite suppressants, as well as in the treatment of obesity or diabetes.

The oligomeric compounds ISIS Number 327901 and ISIS Number 340927 both targeting mir-143, ISIS Number 327892 and ISIS Number 340926 both targeting mir-131/mir-9, and ISIS Number 327878 and ISIS Number 345349 both targeting mir-203 were also tested in db/db mice. Although treatment of db/db mice with the gapmer compounds targeting mir-143, mir-203 or mir-131/mir9 resulted in an approximately 2-fold increase in liver transaminases in db/db mice, the uniform 2'-MOE oligomeric compounds targeting mir-143, mir-203 or mir-131/mir-9 were not found to cause liver toxicity in db/db mice, as assessed by weekly measurements of plasma transaminase levels.

Additional oligomeric compounds targeting miRNAs were studied in ob/ob mice. Six week old ob/ob mice were treated (dose =25 mg/kg, twice weekly for four weeks) with uniform 2'-MOE and gapmer oligomeric compounds targeting mir-143, mir-23b, mir-221, let-7a, and mir-29b, and compared to saline treated animals or animals treated with ISIS 342672 (SEQ ID NO: 789), a uniform 2'-MOE scrambled negative control oligomeric compound bearing 13 base mismatches to mir-143. Expression levels were normalized against the U6 RNA and the expression levels of saline treated animals were set at 100%. Fed plasma samples were taken bi-weekly by tail bleed, and plasma levels of liver transaminases, cholesterol, triglycerides, free fatty acids and glucose were assessed, with the tail bleed on week three taken under fasting conditions. Ob/ob mice were treated with ISIS Numbers 327901 and 340927, the uniform 2'-MOE and gapmer oligomeric compounds, respectively, targeting mir-143 are described above. Additionally, ob/ob mice were also treated with the following compounds: ISIS Number 327889 (SEQ ID NO: 307), a phosphorothioate uniform 2'-MOE oligomeric compound, and ISIS Number 340925 (SEQ ID NO: 307), a 2'-MOE 5-10-8 gapmer oligomeric compound, each targeting mir-23b; ISIS Number 327919 (SEQ ID NO: 337), a uniform 2'-MOE oligomeric compound, and ISIS Number 345384 (SEQ ID NO: 337), a phosphorothioate 2'-MOE 5-10-8 gapmer oligomeric compound, each targeting mir-221; ISIS Number 327903 (SEQ ID NO: 321), a uniform 2'-MOE oligomeric compound, and ISIS Number 345370 (SEQ ID NO: 321), a phosphorothioate 2'-MOE 5-10-7 gapmer oligomeric compound, each targeting let-7a; and ISIS Number 327876 (SEQ ID NO: 294), a uniform 2'-MOE oligomeric compound, and ISIS Number 345347 (SEQ ID NO: 294), a phosphorothioate 2'-MOE 5-10-8 gapmer oligomeric compound, each targeted to mir-29b-1.

Ob/ob mice treated with the gapmer compounds ISIS 340925 and ISIS 345384, targeting mir-23b and mir-221, respectively, exhibited reductions in plasma glucose levels in the fed state

at weeks two and four, as compared to untreated mice, or mice treated with control compounds. Furthermore, mice treated with ISIS 340925 exhibited a decrease in triglycerides in the fourth week. Ob/ob mice treated with ISIS 340925 did not exhibit an increase in plasma transaminases at weeks two or four. Thus, the oligomeric compounds ISIS Numbers 340925 and 345384 may be useful as agents for the treatment of obesity and/or diabetes.

In addition, Northern blot analyses were performed to quantitate the expression of mir-23b in kidney samples of ob/ob mice treated with oligomeric compounds of the present invention. To detect the mir-23b target, a target-specific DNA oligonucleotide probe with the sequence GTGGTAATCCCTGGCAATGTGAT (SEQ ID NO: 307) was synthesized by IDT (Coralville, IA). The oligo probes were 5' end-labeled with T4 polynucleotide kinase with (γ-^{32}P) ATP (Promega). The mir-23b specific DNA oligonucleotide probe was used to detect expression levels of the mir-23b miRNA in ob/ob mice treated (twice weekly at 25 mg/kg) with ISIS Numbers 327889, the uniform 2'-MOE oligomeric compound, or ISIS Number 340925, the 5-10-8 gapmer compound, both targeted to mir-23b, versus saline treated animals or animals treated with a control oligomeric compound, ISIS Number 116847 (CTGCTAGCCTCTGGATTTGA; SEQ ID NO: 844), a uniform 5-10-5 2'-MOE gapmer targeting an unrelated gene, PTEN. Expression levels were normalized against the U6 RNA and the expression levels of saline treated animals were set at 100%. Most notably, in kidney samples from ob/ob mice treated with ISIS Number 327889, the uniform 2'-MOE oligomeric compound targeted to mir-23b exhibited a nearly 64% decrease in *in vivo* expression levels of the mir-23b miRNA. In kidney samples from mice treated with the gapmer oligomeric compound targeting mir-23b, ISIS Number 340925, a 41% reduction in *in vivo* expression levels of the mir-23b miRNA was observed.

As described, *supra*, the C57BL/6 mouse strain is reported to be susceptible to hyperlipidemia-induced atherosclerotic plaque formation, and when these mice are fed a high-fat diet, they develop diet-induced obesity (DIO). Accordingly, the DIO mouse model is useful for the investigation of obesity and development of agents designed to treat these conditions. In one embodiment of the present invention, oligomeric compounds targeting miRNAs were tested in the DIO model. Normal C57/BL6 male mice were fed a high fat diet (40% fat, 41% carbohydrate, 18% protein) for 12 weeks before the study began. DIO mice were then randomized by weight and insulin values. Initial body fat composition was determined by Dual X-ray Absorptiometry (DEXA) Scan. DIO mice were then subcutaneously injected with oligomeric compounds of the invention at a dose of 25 mg/kg, twice weekly. DIO mice were treated with oligomeric compounds ISIS Numbers 327901 and 340927 targeting mir-143, ISIS

Numbers 327892 and 340926 targeting mir-131/mir-9, ISIS Numbers 327878 and ISIS Number 345349 targeting mir-203, and ISIS Numbers 327889 and 340925, targeting mir-23b. As negative controls, "scrambled" oligomeric compounds were also designed: ISIS Number 342672 contains 13 mismatches with respect to the mature mir-143 miRNA; ISIS Number 353607 (ACTAGTTTTTCTTACGTCTGA; herein incorporated as SEQ ID NO: 845) is a phosphorothioate 5-10-6 2'-MOE gapmer oligomeric compound containing 12 mismatches with respect to mir-131/mir-9; ISIS Number 353608 (CTAGACATTAGCTTTGACATCC; herein incorporated as SEQ ID NO: 846) is a phosphorothioate 5-10-7 2'-MOE gapmer oligomeric compound containing 16 mismatches with respect to mir-203. DEXA scans were also performed at weeks 0, 3 and 5 after treatment with the oligomeric compounds to assess the fat mass to lean mass ratio. The effects of target inhibition on levels of plasma glucose and insulin, liver transaminases, cholesterol and triglycerides, were also assessed weekly by tail bleed, and after the treatment period, mice were sacrificed and liver and kidney heart, as well as white adipose tissue (WAT) tissues collected. The mRNA expression levels of the Glut4, aP2, HSL and PPARγ marker genes are also assessed. Treatment of DIO mice with the uniform 2'-MOE oligomeric compounds ISIS 327901 targeting mir-143, ISIS 327892 targeting mir-131/mir9, ISIS 327878 targeting mir-203, and ISIS 327889 targeting mir-23b did not appear to cause liver toxicity in these mice as assessed by weekly measurements of plasma transaminase levels. Similarly, the gapmer oligomeric compounds ISIS 340927 targeting mir-143, and ISIS 340926 targeting mir-131/mir-9, 340925 did not cause significant increases in liver toxicity, and the gapmer compound ISIS 340925 targeting mir-23b caused only an approximately 2-fold increase in the liver transaminase AST. Interestingly, the gapmer compounds ISIS Numbers 340927 targeting mir-143, 340926 targeting mir-131/mir-9, 345349 targeting mir-203, and 340925, targeting mir-23b were all effective at reducing insulin levels at the two and four week time points, as compared to saline-treated control mice. Furthermore, some improvement in body composition (a reduction in body weight and fat mass) was observed. These data from the DIO model suggest that oligomeric compounds targeting mir-143, mir-131/mir-9, mir-203 and mir-23b may be useful as agents for the treatment of obesity and/or diabetes.

Having the information disclosed herein, one of ordinary skill in the art would comprehend that of other classes of inhibitors targeting mir-143, mir-209, mir-131/mir-9 and mir-23b miRNAs, such as antibodies, small molecules, and inhibitory peptides, can be assessed for their effects on the physiological indicators of diseases in *in vivo* models, and these inhibitors can be developed for the treatment, amelioration or improvement of physiological conditions associated with a particular disease state or condition. Such inhibitors are envisioned as within

the scope of the instant invention.

Example 24: Effects of oligomeric compounds on cell cycling

Cell cycle assay:

Cell cycle regulation is the basis for various cancer therapeutics. Cell cycle checkpoints are responsible for surveillance of proper completion of certain steps in cell division such as chromosome replication, spindle microtubule attachment and chromosome segregation, and it is believed that checkpoint functions are compromised in some cancerous cells. Furthermore, because the shift from quiescence to an actively growing state as well as the passage through mitotic checkpoints are essential transitions in cancer cells, most current chemotherapy agents target dividing cells. For example, by blocking the synthesis of new DNA required for cell division, an anticancer drug can block cells in S-phase of the cell cycle. These chemotherapy agents impact many healthy organs as well as tumors. In some cases, a cell cycle regulator will cause apoptosis in cancer cells, but allow normal cells to undergo growth arrest and therefore remain unaffected. Loss of tumor supressors such as p53 sensitizes cells to certain anticancer drugs; however, cancer cells often escape apoptosis. Further disruption of cell cycle checkpoints in cancer cells can increase sensitivity to chemotherapy while allowing normal cells to take refuge in G1 and remain unaffected. A goal of these assays is to determine the effects of oligomeric compounds on the distribution of cells in various phases of the cell cycle.

In some embodiments, the effects of several oligomeric compounds of the present invention were examined in the normal human foreskin fibroblast BJ cell line, the mouse melanoma cell line B16-F10 (also known as B16 cells), as well as the breast carcinoma cell line, T47D. These cell lines can be obtained from the American Type Culture Collection (Manassas, VA). BJ cells were routinely cultured in MEM high glucose with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate and supplemented with 10 % fetal bovine serum, 0.1 mM non-essential amino acids and 1.0 mM sodium pyruvate (all media and supplements from Invitrogen Life Technologies, Carlsbad, CA). B16-F10 cells were routinely cultured in DMEM high glucose (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA). T47D cells were cultured in DMEM High glucose media (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum. Cells were routinely passaged by trypsinization and dilution when they reached 80 to 90% confluence. Cells were plated on collagen-coated 24-well plates (Falcon-Primaria #3047, BD Biosciences, Bedford, MA) at approximately 50,000 cells per well and allowed to attach to wells overnight.

As a negative control, a random-mer oligomeric compound, 20 nucleotides in length, ISIS 29848 (SEQ ID NO: 737) was used. In addition, a positive control, ISIS 183891 (CCGAGCTCTCTTATCAACAG; herein incorporated as SEQ ID NO: 847) was included; ISIS 183891 targets kinesin-like 1 (also known as Eg5) and inhibits cell cycle progression. Eg5 is known to induce apoptosis when inhibited. ISIS 29248 and ISIS 183891 are chimeric oligomeric compounds ("gapmers") 20 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide "wings" (a "5-10-5 gapmer"). The wings are composed of 2'-methoxyethoxy (2'-MOE) nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the compound. All cytidine residues are 5-methylcytidines. ISIS 340348 (CTACCTGCACGAACAGCACTTT; herein incorporated as SEQ ID NO: 848) is a uniform 2'-MOE phosphorothioate oligomeric compound targeting the mir-93 miRNA, and ISIS 340365 (TACTTTATATAGAACACAAG; herein incorporated as SEQ ID NO: 849) is a 5-10-5 gapmer phosphorothioate oligomeric compound targeting the mir-92-2 miRNA.

Oligomeric compounds were mixed with LIPOFECTIN™ (Invitrogen Life Technologies, Carlsbad, CA) in OPTI-MEM™ (Invitrogen Life Technologies, Carlsbad, CA) to acheive a final concentration of 150 nM of oligomeric compound and 4.5 μg/ml LIPOFECTIN™. Before adding to cells, the oligomeric compound, LIPOFECTIN™ and OPTI-MEM™ were mixed thoroughly and incubated for 0.5 hrs. The medium was removed from the plates and each well was washed in 250 μl of phosphate-buffered saline. The wash buffer in each well was replaced with 250 μL of the oligomeric compound/OPTI-MEM™/LIPOFECTIN cocktail. Control cells received LIPOFECTIN™ only. The plates were incubated for 4 hours at 37° C, after which the medium was removed. 100 μl of full growth medium was added to each well. After 72 hours, routine procedures were used to prepare cells for flow cytometry analysis and cells were fixed with ethanol and stained with propidium iodide to generate a cell cycle profile using a flow cytometer. The cell cycle profile was analyzed with the ModFit program (Verity Software House, Inc., Topsham ME).

Fragmentation of nuclear DNA is a hallmark of apoptosis and produces an increase in cells with a hypodiploid DNA content. Cells with a hypodiploid DNA content are categorized as "subG1." The cells in the G1, G2/M and S phases are considered to be cycling, and cells in the subG1 and aneuploid categories are considered to have left the cell cycle. An increase in cells in G1 phase is indicative of a cell cycle arrest prior to entry into S phase; an increase in cells in S phase is indicative of cell cycle arrest during DNA synthesis; and an increase in cells in the

G2/M phase is indicative of cell cycle arrest just prior to or during mitosis. Data are are shown in Table 29 and expressed as percentage of cells in each phase of the cell cycle.

Table 29

Effects of oligomeric compounds targeting miRNAs on cell cycling

ISIS #	SEQ ID #	Pri-miRNA	SubG1	G1	S	G2/M	aneuploid
UTC	N/A	N/A	8.1	59.6	27.5	12.9	7.3
ISIS-29848 n-mer	737	N/A	9.6	57.8	26.5	15.6	12
ISIS-183891 Positive control	847	Kinesin-like 1/Eg5	20.8	33.1	39.2	27.6	11.5
327878	296	mir-203	17.3	39.1	40.8	20	11.9
327888	306	mir-108-1	13.3	53.7	29.5	16.7	12.9
327889	307	mir-23b	8.2	53.1	32.5	14.4	10.5
327901	319	mir-143	12	34.7	44.9	20.3	13.6
327902	320	mir-192-1	10.6	50.7	33.9	15.3	13.4
327903	321	let-7a-3	11	53.7	30.9	15.4	13.4
327904	322	mir-181a	8.6	54.4	29.5	16.2	15.6
327905	323	mir-205	8.5	56.9	28.1	15	14.7
327906	324	mir-103-1	15.2	46.1	33	20.9	15.8
327907	325	mir-26a	17.8	49.5	32.8	17.6	17.8
327908	326	mir-33a	5.6	55.4	29.2	15.3	13.1
327909	327	mir-196-2	7.9	52.6	30.1	17.3	16.3
327910	328	mir-107	9.3	49.5	33	17.5	13.1
327911	329	mir-106	10.9	49.9	30.1	20	16.5
327914	332	mir-130a	8.5	55.8	28.9	15.3	16.2
327919	337	mir-221	10.8	54.3	30.3	15.4	16
327922	340	mir-19b-2	10	50.4	30.7	18.9	16.8
327928	346	mir-29a-1	6.6	56	27.9	16	15.9
327933	351	mir-145	10.2	49.6	31.3	19.1	15.9
327934	352	mir-213	6.6	54.4	28.2	17.4	17
327941	359	mir-181b	8.2	57.2	29.9	12.9	15.8
327951	369	mir-15a-1	4.3	60.9	24.8	14.3	16.7
328342	451	mir-203	4.8	62.3	24.9	12.8	15.2
328362	471	mir-108-1	9.1	51.2	33.6	15.1	12.9
328364	473	mir-23b	1.9	61.5	24.2	14.3	15.1
328382	491	mir-143	2.9	59.8	25.7	14.4	14.8
328388	497	let-7a-3	4.0	57.5	28	14.6	14.5
328394	503	mir-181a	2.4	59.5	24.5	16	18.3
328396	505	mir-205	4.6	56.8	28.2	15	19.8
328419	528	mir-221	6.0	51.2	32.5	16.3	17.9
328423	532	mir-19b-2	4.9	52.9	32.4	14.8	15.3
328424	533	mir-19b-2	3.1	61.9	23.7	14.4	16.9
328436	545	mir-29a-1	3.5	59.2	26.9	13.9	17.4
328644	553	mir-145	7.2	58.4	27.6	14	17.5
328691	600	mir-145	7.7	60.5	24.4	15.1	16.6
328697	606	mir-181b	2.4	57.6	26.4	16	13.5
328773	682	mir-15a-2	2.7	56.4	26.9	16.7	11.7
340348	848	mir-93	14.1	53.9	31.8	14.3	12.3
340365	849	mir-92-2	4.3	55.2	29.4	15.4	18.3

From these data, it is evident that treatment with the oligomeric compounds targeting mir-143, ISIS Number 327901 (SEQ ID NO: 319); mir-203, ISIS Number 327878 (SEQ ID NO:

296); mir-103-1, ISIS Number 327906 (SEQ ID NO: 324); mir-106, ISIS Number 327911 (SEQ ID NO: 329); and mir-145, ISIS Number 327933 (SEQ ID NO: 351) resulted in an increased percentage of cells in the G2/M phase, indicating that these oligomeric compounds arrest or delay the cell cycle at or just prior to mitosis, potentially activating a mitotic checkpoint.

Treatment with the oligomeric compounds targeting mir-26a, ISIS Number 327907 (SEQ ID NO: 325); mir-205, ISIS Number 328396 (SEQ ID NO: 505); mir-181a, ISIS Number 328394 (SEQ ID NO: 503); and mir-92-2, ISIS Number 340365 (SEQ ID NO: 849) resulted in higher than average percentages of aneuploid cells, indicating that these oligomeric compounds interfere with proper chromosome segregation.

Treatment with the oligomeric compounds targeting mir-203, ISIS Number 327878 (SEQ ID NO: 296); mir-103-1, ISIS Number 327906 (SEQ ID NO: 324); mir-26a, ISIS Number 327907 (SEQ ID NO: 325); and mir-93, ISIS Number 340348 (SEQ ID NO: 848) resulted in an increased percentage of cells with hypodiploid DNA content (SubG1 phase) indicating that the oligomeric compound treatment may induce apoptotic events.

The effects of several oligomeric compounds of the present invention were also examined in the HeLa and A549 human carcinoma cell lines, both of which can be obtained from the American Type Culture Collection (Manassas, VA).

In some embodiments, HeLa cells were plated on collagen-coated 24-well plates at 50,000-60,000 cells per well, and allowed to attach to wells overnight. In some embodiments, HeLa cells were synchronized by double thymidine block (cells were washed three times with PBS, then grown in 10% FBS containing 2mM thymidine; then 19 hours later, cells were washed three times in PBS, 10% FBS for 9 hours; cells were then incubated in 10% FBS, 2mM thymidine for 15 hours; then washed three times with PBS, 10% FBS and samples were taken every two hours over a 16 hour period). A portion of each time sample was fixed with ethanol and treated with propidium iodide and subjected to FACs analysis for determination of the percentage of cells in each phase of the cell cycle. Distinctive peaks were observed for G0-, S-, Early G2/M-, Late G2/M-, and G1-phases of the cell cycle at 0-, 4-, 6-, 8-, and 12-hours, respectively, indicating that the cells were synchronized. HeLa cells treated with 10μM cisplatin or 100 ng/ml nocodazole were used as controls for G1-phase and late G2/M-phases, respectively. From the remaining portion of each of these time samples, total RNA was isolated and used to assess the expression of cell cycle marker mRNAs using the real-time RT-PCR methods and/or used to screen microarrays to assess the expression of miRNAs over the course of the cell cycle. It was observed that several miRNAs are expressed in a cell-cycle-dependent manner. Shown in Table 30 are the mRNA levels of the E2F1 transcription factor and topoisomerase 2A (Top2A),

which vary over the course of the cell cycle and can be used for comparison to the experimental groups for the confirmation of cell cycle phase. Data are an average of three trials.

Table 30

Expression levels of cell cycle markers

treatment	E2F1 mRNA	Top2A mRNA
10 uM cisplatin	102	15
100 ng/ml nocodazole	23	176
0 hrs (G0-phase)	100	100
4 hrs (S-phase)	81	105
6 hrs (early G2/M-phase)	39	221
8 hrs (late G2/M-phase)	50	254
12 hrs (G1-phase)	61	124

In some embodiments, HeLa cells were also treated with oligomeric compounds targeting miRNAs. As described above, oligomeric compounds were mixed with LIPOFECTIN™ in OPTI-MEM™ (Invitrogen Life Technologies, Carlsbad, CA) to a final concentration of 150 nM of oligomeric compound and 6 μg/ml LIPOFECTIN™. Before adding to cells, the oligomeric compound, LIPOFECTIN™ and OPTI-MEM™ were mixed thoroughly and incubated for 0.5 hrs. The medium was removed from the plates. Each well was washed in 250 μl of PBS. The wash buffer in each well was replaced with 250 μL of the oligomeric compound/OPTI-MEM™/LIPOFECTIN cocktail. Control cells received LIPOFECTIN™ only. The plates were incubated for 4 hours at 37° C, after which the medium was removed. 1000 μl of full growth medium was added to each well. After 24 hours (Table 31) or 48 hours (Table 32), cells were prepared for flow cytometry analysis to generate a cell cycle profile. The cell cycle profile was analyzed with the ModFit program (Verity Software House, Inc., Topsham ME).

The random-mer ISIS 29848 (SEQ ID NO: 737) was used as a negative control, and ISIS 183891 (SEQ ID NO: 847), targeting kinesin-like 1/Eg5, was included as a positive control. Results of these experiments are shown in Tables 31 and 32. Data are expressed as percentage of cells in each phase relative to the untreated control (UTC); values above 100 are considered to indicate a delay or arrest in that phase of the cell cycle. Table 31 shows the results from cells sampled 24 hours after oligomeric compound treatment, and Table 32 shows the results from cells sampled 48 hours after oligomeric compound treatment. In some cases, the same oligomeric compound was tested in repeated experiments.

Table 31

Effects of oligomeric compounds targeting miRNAs on cell cycling (24 hours)

Pri-miRNA	ISIS #		% cells in cell cycle phase

		SEQ ID #	subG1	G1	S	G2/M	aneuploid
UTC	N/A	N/A	100	100	100	100	100
n-mer	29848	737	120	116	81	108	76
Kinesin-like 1/Eg5	183891	847	251	21	109	231	95
collagen, type I, alpha 1/ hypothetical miRNA-144	338797	624	197	101	79	148	193
hypothetical miRNA-039	338666	493	235	123	63	158	102
hypothetical miRNA-111	328111	413	62	127	75	99	50
hypothetical miRNA-111	338750	577	107	148	76	97	166
hypothetical miRNA-142	328115	417	177	90	87	147	59
hypothetical miRNA-154	328119	421	75	100	94	112	83
hypothetical miRNA-154	328724	633	155	91	90	135	197
hypothetical miRNA-179	328749	658	312	126	82	110	138
hypothetical miRNA-179	328780	689	124	96	87	136	149
hypothetical miRNA-181	328136	438	330	125	81	88	51
hypothetical miRNA-181	338833	660	232	150	56	142	185
let-7a-3	327903	321	118	92	104	106	98
let-7a-3	328388	375	120	110	83	115	85
mir-100-1	327957	497	197	91	88	145	66
mir-100-1	328707	616	188	36	93	195	166
mir-103-1	327906	324	228	153	47	107	65
mir-103-1	328397	506	134	93	86	142	91
mir-106	327911	329	158	130	62	122	104
mir-106	328403	512	284	70	85	197	53
mir-106	328403	512	189	86	75	179	82
mir-107	327910	328	174	154	42	118	73
mir-108-1	328362	471	114	101	87	126	66
mir-10a	327949	367	194	82	84	172	68
MiR-125a, Mouse	341787	852	221	113	75	144	165
mir-127, Mouse	341788	853	303	154	54	140	114
mir-130b	328687	596	231	80	98	131	149
mir-130b	338769	596	188	171	61	103	133
mir-131-2/mir-9	327892	310	153	86	111	103	80
mir-131-2/mir-9	328369	310	84	100	88	125	71
mir-131-2/mir-9	340926	478	286	98	91	121	83
mir-133b	338713	540	93	152	72	101	187
mir-141	338741	568	157	141	73	112	166
mir-143	327901	319	108	101	94	110	90
mir-143	328382	491	81	118	76	116	78
mir-143	328382	491	226	102	80	144	202
mir-143	340927	319	118	121	75	111	88
mir-143	340927	319	131	128	71	106	87
mir-145	327933	351	192	102	83	131	92
mir-145	327933	351	190	90	91	140	47
mir-145	328644	553	71	113	84	109	68
mir-145	345395	351	247	54	82	222	77
mir-149, Mouse	341785	854	125	152	92	53	158
mir-152	328727	636	245	133	81	105	161
mir-152	338809	636	106	159	82	69	210
mir-16-3	327877	295	154	107	66	159	62
mir-17/mir-91	327885	303	151	129	63	121	55
mir-181a-1	327904	322	114	99	102	99	89
mir-182	328744	653	229	31	108	167	111
mir-182	338826	653	145	148	79	90	138
mir-192-1	327902	320	178	57	106	176	66
mir-192-1	327902	320	175	44	121	163	98
mir-192-1	328383	492	314	55	82	222	92

mir-192-1	328383	492	289	63	97	183	98
mir-192-1	338665	340	173	85	76	175	193
mir-19b-2	327922	492	131	97	96	114	104
mir-19b-2	328424	533	60	110	85	112	74
mir-203	327878	296	124	96	94	122	73
mir-203	328342	451	192	33	95	238	67
mir-205	327905	323	144	99	88	129	50
mir-205	327905	323	149	94	95	121	98
mir-205	328396	505	97	94	87	139	88
mir-205	338678	505	162	122	75	131	202
mir-211	327946	364	225	90	84	156	43
mir-211	328674	583	564	125	93	84	69
mir-211	338756	583	137	147	75	99	166
mir-213/mir-181a-2	327934	352	278	87	85	160	55
mir-213/mir-181a-2	327934	352	204	118	66	137	77
mir-213/mir-181a-2	328647	556	140	101	92	119	140
mir-216	327956	374	120	124	68	120	61
mir-216	328759	668	239	88	78	168	184
mir-22	327896	314	121	83	103	128	65
mir-22	328374	483	198	54	115	162	97
mir-220	327944	362	165	85	110	111	50
mir-221	327919	337	85	92	103	109	96
mir-221	328419	528	87	109	79	124	77
mir-23a	338836	663	153	185	53	105	150
mir-23b	327889	307	122	104	102	87	82
mir-23b	340925	307	151	103	89	117	73
mir-26a-1	327907	325	224	119	77	111	75
mir-26a-1	345373	325	196	66	94	176	68
mir-29b-1	327876	294	103	98	104	95	66
mir-29b-1	327876	294	149	93	92	131	75
mir-29b-1	328337	446	107	106	88	113	104
mir-29b-1	328337	446	99	108	88	109	64
mir-29b-2	328339	448	235	77	102	143	61
mir-29c	338690	517	149	124	78	123	194
mir-30a	328084	585	381	43	104	163	101
mir-30b	328676	585	139	99	86	134	169
mir-30b	338758	743	113	129	81	108	190
mir-30d	328421	530	288	47	105	200	70
mir-33a	327908	326	138	98	99	106	114
mir-92-1	327897	315	143	114	80	115	69
mir-92-1	327897	315	180	128	74	100	54
mir-92-2	340365	849	109	125	71	114	84
mir-95 (Mourelatos)	340350	855	218	183	54	104	94
mir-96	338637	464	88	170	70	84	188

Table 32

Effects of oligomeric compounds targeting miRNAs on cell cycling (48 hours)

Pri-miRNA	ISIS #	SEQ ID #	% cells in cell cycle phase				
			subG1	G1	S	G2/M	aneuploid
UTC	N/A	N/A	100	100	100	100	100
n-mer	29848	737	86	87	121	117	109
Kinesin-like 1/Eg5	183891	847	173	19	124	331	72
collagen, type I, alpha 1/ hypothetical miRNA-144	338797	624	813	66	124	168	175
hypothetical miRNA-039	338666	493	1832	44	136	217	125
hypothetical miRNA-111	328111	413	371	84	126	119	90

hypothetical miRNA-111	338750	577	201	99	101	103	190
hypothetical miRNA-142	328115	417	195	92	114	107	86
hypothetical miRNA-154	328119	421	767	75	145	124	81
hypothetical miRNA-154	328724	633	653	70	134	140	155
hypothetical miRNA-179	328749	658	962	37	129	246	65
hypothetical miRNA-179	328780	689	917	83	130	110	133
hypothetical miRNA-181	328136	438	140	83	133	113	85
hypothetical miRNA-181	338833	660	1091	44	106	258	154
let-7a-3	327903	321	74	102	95	98	94
let-7a-3	328388	375	112	99	101	102	126
mir-100-1	327957	497	864	65	169	127	85
mir-100-1	328707	616	1486	46	134	213	155
mir-103-1	327906	324	57	100	98	103	83
mir-103-1	328397	506	74	97	101	109	96
mir-106	327911	329	65	99	96	109	101
mir-106	328403	512	863	61	177	131	85
mir-106	328403	512	108	82	148	106	80
mir-107	327910	328	53	99	91	111	92
mir-108-1	328362	471	87	96	104	108	97
mir-10a	327949	367	773	66	157	138	71
MiR-125a, Mouse	341787	852	707	55	126	197	153
mir-127, Mouse	341788	853	748	76	105	163	116
mir-130b	328687	596	1119	55	174	131	171
mir-130b	338769	596	482	76	116	149	194
mir-131-2/mir-9	327892	310	121	74	150	129	79
mir-131-2/mir-9	328369	310	72	99	95	109	109
mir-131-2/mir-9	340926	478	68	83	120	131	106
mir-133b	338713	540	426	95	104	109	194
mir-141	338741	568	185	100	101	99	170
mir-143	327901	319	93	98	104	103	104
mir-143	328382	491	71	102	92	103	109
mir-143	328382	491	350	83	122	120	133
mir-143	340927	319	95	91	107	121	113
mir-143	340927	319	83	91	107	122	108
mir-145	327933	351	91	76	135	138	86
mir-145	327933	351	438	80	133	123	75
mir-145	328644	553	52	101	101	98	82
mir-145	345395	351	213	51	192	157	87
mir-149, Mouse	341785	854	1148	82	126	116	166
mir-152	328727	636	846	68	152	124	140
mir-152	338809	636	345	86	110	129	157
mir-16-3	327877	295	755	59	152	168	80
mir-17/mir-91	327885	303	456	78	129	133	76
mir-181a-1	327904	322	116	87	126	114	80
mir-182	328744	653	1774	31	78	334	171
mir-182	338826	653	696	61	124	182	137
mir-192-1	327902	320	1176	39	171	208	81
mir-192-1	327902	320	202	44	166	205	87
mir-192-1	328383	492	303	53	217	124	90
mir-192-1	328383	492	940	54	178	150	90
mir-192-1	338665	340	1629	40	89	292	149
mir-19b-2	327922	492	81	96	105	109	91
mir-19b-2	328424	533	89	103	91	101	111
mir-203	327878	296	50	89	119	114	92
mir-203	328342	451	189	55	115	225	107
mir-205	327905	323	719	48	194	150	67
mir-205	327905	323	100	78	143	122	99
mir-205	328396	505	88	89	114	119	129
mir-205	338678	505	1158	81	78	188	179

mir-211	327946	364	431	72	150	129	76
mir-211	328674	583	1663	69	160	109	134
mir-211	338756	583	311	90	121	100	169
mir-213/mir-181a-2	327934	352	752	62	156	152	92
mir-213/mir-181a-2	327934	352	155	66	148	155	117
mir-213/mir-181a-2	328647	556	589	69	153	118	136
mir-216	327956	374	184	91	106	121	110
mir-216	328759	668	1744	50	31	343	148
mir-22	327896	314	886	55	140	194	66
mir-22	328374	483	787	65	157	143	71
mir-220	327944	362	490	75	129	144	78
mir-221	327919	337	104	80	122	139	104
mir-221	328419	528	83	99	96	107	112
mir-23a	338836	663	811	52	152	169	165
mir-23b	327889	307	133	78	137	130	101
mir-23b	340925	307	89	87	130	109	93
mir-26a-1	327907	325	116	92	111	115	94
mir-26a-1	345373	325	116	75	132	145	119
mir-29b-1	327876	294	41	87	120	119	100
mir-29b-1	327876	294	251	76	141	126	69
mir-29b-1	328337	446	66	92	105	119	108
mir-29b-1	328337	446	662	73	143	135	74
mir-29b-2	328339	448	678	73	153	123	92
mir-29c	338690	517	413	91	110	112	190
mir-30a	328084	585	1028	20	168	241	57
mir-30b	328676	585	366	86	118	118	172
mir-30b	338758	743	267	103	99	92	153
mir-30d	328421	530	1103	30	202	198	64
mir-33a	327908	326	61	99	98	105	93
mir-92-1	327897	315	134	100	103	95	84
mir-92-1	327897	315	125	94	114	105	63
mir-92-2	340365	849	71	99	94	109	129
mir-95 (Mourelatos)	340350	855	1144	76	126	134	125
mir-96	338637	464	239	90	109	117	210

Several oligomeric compounds were observed to result in an arrest or delay of the cell cycle, in some cases correlating with a cell-cycle-dependent expression profile as determined by miRNA microarray analysis.

For example, from these data, it was observed that treatment of HeLa cells with oligomeric compounds (MOE-gapmers and fully modified MOEs) targeting miRNAs caused an increase in the percentage of cells exhibiting a subG1-phase or aneuploid DNA content, indicating aberrant chromosome segregation. Treatment with oligomeric compounds ISIS Number 338797 (SEQ ID NO: 624) targeted to hypothetical miRNA-144, ISIS Number 338833 (SEQ ID NO: 660) targeted to hypothetical miRNA-181, and ISIS Number 328759 (SEQ ID NO: 668) targeted to mir-216, each appeared to cause an induce chromosome missegregation events at both the 24-hour and 48-hour timepoints. Thus, these compounds may be useful in triggering a checkpoint arrest in rapidly dividing cells, potentially useful in the treatment of hyperproliferative disorders such as cancer.

It was also observed that other oligomeric compounds (MOE-gapmers and fully modified MOEs) targeting miRNAs appeared to induce an arrest or delay in the G1-, S-, or G2/M-phases of the cell cycle. By miRNA microarray analysis, expression levels of the mir-205 miRNA were observed to increase in the S-and G1-phases of the cell cycle in HeLa cells. Treatment of HeLa cells with the oligomeric compound ISIS Number 327905 (SEQ ID NO: 323), targeting the mir-205 miRNA, was observed to arrest or delay the cell cycle in S-phase at the 48-hour time point, suggesting that the mir-205 miRNA may play a role in regulating DNA replication. It was also observed that treatment of HeLa cells with the oligomeric compound ISIS Number 338678 (SEQ ID NO: 505), targeted to the mir-205 pri-miRNA, resulted in an arrest or delay primarily in the G2/M-phase of the cell cycle, suggesting that this oligomeric compound may interfere with processing of the miRNA precursor into a mature miRNA, which appears to have an impact on mitosis.

Treatment of HeLa cells with oligomeric compounds ISIS Number 327892 (SEQ ID NO: 310), targeting mir-131/mir-9, and ISIS Number 327934 (SEQ ID NO: 352), targeting mir-213/mir-181a-2, was observed to arrest or delay the cell cycle in G2/M-and S-phases at the 48-hour time point, suggesting that the mir-131/mir-9 and mir-213/mir-181a-2 miRNAs are involved in regulating DNA replication and entry into mitosis.

Treatment of HeLa cells with oligomeric compound ISIS Number 345373 (SEQ ID NO: 325), targeting miR-26a-1, was observed to arrest or delay cells mainly in the G2/M-phase at 24 hrs after oligonucleotide-treatment, and at 48 hrs after oligonucleotide-treatment to arrest or delay cells mainly in S-phase of the cell cycle, suggesting that miR-26a-1 is involved in mitosis and that cells making it through a first round of cell division may harbor errors that cause them to arrest during a new round of DNA replication.

By miRNA microarray analysis, expression levels of the mir-145 miRNA were observed to increase in the G2/M-phase of the cell cycle in HeLa cells, and treatment of HeLa cells with the oligomeric compounds ISIS Number 327933 (SEQ ID NO: 351), a uniform 2'-MOE compound, and ISIS Number 345395 (SEQ ID NO: 351), a chimeric 2'-MOE gapmer compound, both targeting the mir-145 miRNA, were observed to arrest or delay the cell cycle in G2/M-phase at the 24-hour time point and at subG1-phase at the 48-hour time point, suggesting that the mir-145 miRNA plays a role in mitosis and that cells making it through a first round of cell division may harbor errors that cause them to arrest before a new round of DNA replication.

By miRNA microarray analysis, expression levels of the mir-192-1 miRNA were observed to increase in the G2/M-phase of the cell cycle in HeLa cells, and treatment of HeLa cells with the oligomeric compounds ISIS Number 327902 (SEQ ID NO: 320), a uniform 2'-

MOE compound, and ISIS Number 328383 (SEQ ID NO: 492), a chimeric 2'-MOE gapmer compound, targeted against the mir-192-1 miRNA and the mir-192-1 precursor, respectively, were observed to arrest or delay the cell cycle in the G2/M-phase at 24-hours after oligonucleotide treatment, and at both the S- and G2/M-phases at the 48-hour time point, suggesting that the mir-192 miRNA is involved in mitosis, and that cells making it through a first round of cell division may harbor errors that cause them to arrest during a new round of DNA replication. A uniform 2'-MOE oligomeric compound ISIS Number 338665 targeting the mir-192-1 precursor was also observed to induce a G2/M-phase arrest at both time points.

Treatment of HeLa cells with the oligomeric compound ISIS Number 328744 (SEQ ID NO: 653), targeting the mir-182 miRNA, was observed to arrest or delay the cell cycle in G2/M-phase at 48-hours after oligonucleotide treatment, suggesting that the mir-182 miRNA plays a role in regulating mitosis.

Treatment of HeLa cells with the oligomeric compound ISIS Number 328421 (SEQ ID NO: 530), targeting miR-30d was also observed to arrest or delay cells mainly in the G2/M-phase at the 24-hour time point and at both the S- and G2/M-phases at the 48-hour time point after oligonucleotide treatment, suggesting that the mir-30d miRNA is involved in mitosis, and that a cell division error arising from the first round of division may allow cells to pass through mitosis and initiate a second round of division, but then a cell cycle checkpoint is set off before the cells are able to complete DNA synthesis.

Treatment of HeLa cells with the oligomeric compound ISIS Number 328403 (SEQ ID NO: 512), targeting mir-106 was also observed to arrest or delay cells in the G2/M-phase at the 24-hour time point and at both the S- and G2/M-phases at the 48-hour time point after oligonucleotide treatment, suggesting that the mir-106 miRNA is involved in mitosis, and that a cell division error arising from the first round of division may allow cells to pass through mitosis and initiate a second round of division, but then a cell cycle checkpoint is set off before the cells are able to complete DNA synthesis. Interestingly, the cell cycle regulatory transcription factor E2F1 mRNA is reported to be a target of the mir-106 miRNA (Lewis et al., *Cell,* **2003**, *115*, 787-798).

Treatment of HeLa cells with the oligomeric compound ISIS Number 328759 (SEQ ID NO: 668), targeting the mir-216 miRNA, was observed to arrest or delay the cell cycle in G2/M-phase at both 24- and 48-hours after oligonucleotide treatment, suggesting that the mir-216 miRNA plays a role in regulating mitosis.

Treatment of HeLa cells with the oligomeric compound ISIS Number 328342 (SEQ ID NO: 451), targeting the mir-203 miRNA, was observed to arrest or delay the cell cycle in G2/M-

phase at both 24- and 48-hours after oligonucleotide treatment, suggesting that the mir-203 miRNA plays a role in regulating mitosis.

Treatment of HeLa cells with the oligomeric compound ISIS Number 328707 (SEQ ID NO: 616), targeting miR-100-1 was also observed to arrest or delay cells mainly in the G2/M-phase at both 24- and 48-hours after oligonucleotide treatment, suggesting that the miR-100-1 miRNA plays a role in regulating mitosis.

Dose responsiveness:

In accordance with the present invention, certain oligomeric compounds targeting miRNAs were selected for dose response studies. Using the cell cycle assay described above, the cell cycle profiles of HeLa or A549 cells treated with varying concentrations of oligomeric compounds of the present invention were assessed.

HeLa cells were treated with 25-, 50-, 100- or 150 nM of the oligomeric compounds ISIS Numbers 327902 (SEQ ID NO: 320) and 328383 (SEQ ID NO: 492), both targeted against mir-192, and ISIS 327905 (SEQ ID NO: 323), targeting mir-205, and ISIS 328403 (SEQ ID NO: 512), targeting mir-106. Cells treated with increasing concentrations of oligomeric compounds were compared to untreated cells, to assess the dose-dependency of the observed delay or arrest. The random-mer ISIS 29848 was used as a negative control. Cells were prepared for flow cytometry 48-hours after oligonucleotide treatment, as described, *supra*. Oligomeric compounds targeted to miRNAs were tested in quadruplicate, and ISIS 29848 was tested in triplicate; data is presented as an average of the replicates. Results of these dose response studies are shown in Table 33, where data are expressed as percentage of cells in each phase.

Table 33

Dose response of oligomeric compounds targeting miRNAs on cell cycling (48 hours)

ISIS #	Dose oligomeric compound	% cells in cell cycle phase				
		SubG1	G1	S	G2/M	Aneuploid
Untreated control (UTC)	25 nM	1.3	56	24	20	12
	50 nM	1.4	56	24	20	14
	100 nM	1.6	57	24	19	11
	150 nM	1.6	57	23	20	15
29848	25 nM	2.0	55	25	20	12
	50 nM	1.5	56	25	19	12
	100 nM	3.2	52	28	20	13
	150 nM	4.2	48	31	21	15
327902	25 nM	1.6	57	23	19	13
	50 nM	2.4	51	30	20	14
	100 nM	3.1	43	30	27	11
	150 nM	6.3	36	36	28	12
327905	25 nM	1.7	57	24	18	12
	50 nM	2.1	50	30	20	12
	100 nM	2.5	46	30	24	14
	150 nM	4.5	38	38	24	12

328383	25 nM	1.9	57	25	18	12
	50 nM	1.3	56	25	18	13
	100 nM	9.3	36	34	30	10
	150 nM	11.8	29	36	34	11
328403	25 nM	1.5	58	24	18	13
	50 nM	1.1	53	27	20	14
	100 nM	3.5	48	29	23	11
	150 nM	8.2	37	40	24	13

From these data, it was observed that 48-hours after treatment of HeLa cells with increasing doses of each of these four oligomeric compounds targeting miRNAs, a dose-responsive delay or arrest resulted, exhibited as an increasing percentage of cells in the S- and G2/M-phases of the cell cycle. Concomittent decreases in the percentage of cells in G1-phase of the cell cycle and increases in the percentage of hypodiploid (subG1) cells were also observed. Likewise, a dose-responsive G2/M delay or arrest was observed in A549 cells treated with 25-, 50-, 100-, or 150 nM of the oligomeric compounds ISIS 327902, ISIS 328383 and ISIS Number 328342.

In a further study, A549 cells were treated with 25-, 50-, 100- or 150 nM of the oligomeric compounds ISIS Numbers 338637 (SEQ ID NO: 464) targeted against mir-96, and ISIS 338769 (SEQ ID NO: 596) targeted against mir-130b, ISIS 338836 (SEQ ID NO: 663) targeted against mir-23a, and ISIS 340350 (SEQ ID NO: 855) targeted against mir-95 (Mourelatos). Cells treated with increasing concentrations of oligomeric compounds were compared to untreated cells, to assess the dose-responsiveness of the observed delay or arrest. The random-mer ISIS 29848 was used as a negative control. Cells were prepared for flow cytometry 24-hours after oligonucleotide treatment. Results of these dose response studies are shown in Table 34, where data are expressed as percentage of cells in each phase relative to the untreated control cells in that phase.

Table 34

Dose response of oligomeric compounds targeting miRNAs on cell cycling (24 hours)

ISIS #	Dose oligomeric compound	% cells in cell cycle phase				
		SubG1	G1	S	G2/M	Aneuploid
29848	25 nM	90	121	86	87	76
	50 nM	91	116	88	93	90
	100 nM	272	125	74	112	116
	150 nM	507	126	71	119	84
338637	25 nM	89	100	99	101	99
	50 nM	86	110	89	107	120
	100 nM	67	126	73	115	146
	150 nM	216	123	66	144	135
338769	25 nM	62	101	94	114	101
	50 nM	82	114	81	122	132
	100 nM	130	124	75	113	157

	150 nM	341	117	71	145	184
338836	25 nM	76	97	103	97	99
	50 nM	232	113	89	98	111
	100 nM	68	117	80	116	153
	150 nM	178	117	69	149	114
340350	25 nM	91	102	100	95	120
	50 nM	158	128	67	126	80
	100 nM	267	125	60	155	107
	150 nM	402	128	40	211	108

From these data, it was observed that 24-hours after treatment of A549 cells with increasing doses of the oligomeric compounds ISIS Numbers 338637 (SEQ ID NO: 464) targeted against mir-96, and ISIS 338769 (SEQ ID NO: 596) targeted against mir-130b, ISIS 338836 (SEQ ID NO: 663) targeted against mir-23a, and ISIS 340350 (SEQ ID NO:855) targeted against mir-95 (Mourelatos), a dose-responsive delay or arrest resulted, exhibited as an increasing percentage of cells in the G2/M-phases of the cell cycle. Concomittent decreases in the percentage of cells in S-phase of the cell cycle and increases in the percentage of hypodiploid (subG1) cells were also observed.

In further studies, additional cell lines were treated with oligomeric compounds targeted against miRNAs to assess the effects of each oligomeric compound on cell cycling. BJ, B16, T47D, and HeLa cells were cultured and transfected as described above. T47D cells are deficient in p53. T47Dp53 cells are T47D cells that have been transfected with and selected for maintenance of a plasmid that expresses a wildtype copy of the p53 gene (for example, pCMV-p53; Clontech, Palo Alto, CA.), using standard laboratory procedures. BJ cells were treated with 200 nM of each oligomeric compound, and T47D, T47Dp53, HeLa, and B16 cells were treated with 150 nM of each oligomeric compound. The human foreskin fibroblast BJ cell line represents a non-cancer cell line, while HeLa, T47D, T47Dp53 cells and the mouse melanoma cell line B16-F10 represent cancerous cell lines. For comparison, oligomeric compounds ISIS 183891 (SEQ ID NO: 847) and ISIS 285717 (TCGGTTCTTTCCAAGGCTGA; herein incorporated as SEQ ID NO: 857), both targeting the kinesin-like 1/Eg5 mRNA, involved in cell cycling, were used as positive controls. The random-mer ISIS 29848 was used as a negative control. Additionally, the oligomeric compounds ISIS Number 25690 (ATCCCTTTCTTCCGCATGTG; herein incorporated as SEQ ID NO: 858) and ISIS Number 25691 (GCCAAGGCGTGACATGATAT; herein incorporated as SEQ ID NO: 859), targeted to nucleotides 3051-3070 and 3085-3104, respectively, of the mRNA encoding the Drosha RNase III (GenBank Accession NM_013235.2, incorporated herein as SEQ ID NO: 860) were also tested. ISIS Number 25690 and ISIS Number 25691 are 5-10-5 2'-MOE gapmer compounds, 20 nucleotides in length, with phosphorothioate internucleoside linkages throughout the oligomeric

compound. All cytidine residues are 5-methylcytidines. Transfections were performed using the methods described herein. Cells were prepared for flow cytometry 48-hours after oligonucleotide treatment. Results of these studies are shown in Table 35, where data are expressed as percentage of cells in each phase relative to the untreated control cells in that phase.

Table 35

Effects of oligomeric compounds targeting miRNAs on cell cycling (48 hours)

Cell line	ISIS #	SEQ ID NO	target	% cells in cell cycle phase				
				Sub G1	G1	S	G2/M	aneuploid
BJ	29848	737	N/A	187	100	99	100	105
B16	29848	737	N/A	143	98	98	110	99
HeLa	29848	737	N/A	403	83	113	106	155
T47D	29848	737	N/A	86	95	113	98	155
T47Dp53	29848	737	N/A	173	121	75	97	93
BJ	183891	847	kinesin-like 1/eg5	422	58	173	287	158
B16	285717	857	kinesin-like 1/eg5	627	72	78	220	178
HeLa	183891	847	kinesin-like 1/eg5	1237	22	95	211	161
T47D	183891	847	kinesin-like 1/eg5	85	55	84	156	161
T47Dp53	183891	847	kinesin-like 1/eg5	351	71	53	189	84
HeLa	25690	858	Drosha, RNAse III	64	119	89	87	140
T47D	25690	858	Drosha, RNAse III	97	97	80	113	140
T47Dp53	25690	858	Drosha, RNAse III	193	97	108	114	144
BJ	25691	859	Drosha, RNAse III	183	94	116	125	209
B16	25691	859	Drosha, RNAse III	316	116	83	99	105
HeLa	25691	859	Drosha, RNAse III	881	53	141	113	203
T47D	25691	859	Drosha, RNAse III	125	94	104	104	203
T47Dp53	25691	859	Drosha, RNAse III	212	130	66	93	95
HeLa	338797	624	hypothetical miRNA-144	144	104	89	115	125
HeLa	338666	493	hypothetical miRNA 039	214	92	98	130	151
HeLa	338833	660	hypothetical miRNA 181	255	87	100	136	136
HeLa	328707	616	mir-100-1	125	103	87	122	140
BJ	328403	512	mir-106	81	102	95	92	114
B16	328403	512	mir-106	112	111	88	99	92
HeLa	328403	512	mir-106	89	125	89	80	175
T47D	328403	512	mir-106	49	104	112	89	175
T47Dp53	328403	512	mir-106	140	114	87	94	89
HeLa	341787	852	MiR-125a, Mouse	324	88	96	145	177
T47D	328687	596	mir-130b	142	101	92	115	169
T47Dp53	338769	596	mir-130b	116	103	87	123	87
B16	327933	351	mir-145	104	109	84	116	130
BJ	345395	351	mir-145	132	100	97	104	115
B16	345395	351	mir-145	147	106	87	115	150
HeLa	345395	351	mir-145	87	108	96	95	139
BJ	328744	653	mir-182	125	94	111	127	158
B16	328744	653	mir-182	153	108	87	110	115
HeLa	328744	653	mir-182	1057	53	110	213	178
T47D	328744	653	mir-182	85	90	87	118	191
T47Dp53	328744	653	mir-182	90	130	59	101	100
BJ	327902	320	mir-192-1	91	99	88	108	82
B16	327902	320	mir-192-1	151	112	88	98	101
HeLa	327902	320	mir-192-1	94	108	96	93	162

T47D	327902	320	mir-192-1	102	75	120	116	162
T47Dp53	327902	320	mir-192-1	155	100	98	102	97
HeLa	338665	492	mir-192-1	322	92	92	142	138
HeLa	328342	451	mir-203	103	96	89	138	96
BJ	327905	323	mir-205	105	100	77	109	102
B16	327905	323	mir-205	142	107	89	106	94
HeLa	327905	323	mir-205	55	108	99	90	164
T47D	327905	323	mir-205	81	97	101	103	164
T47Dp53	327905	323	mir-205	109	112	80	104	103
HeLa	338678	505	mir-205	129	103	94	105	132
BJ	328759	668	mir-216	164	91	117	141	160
B16	328759	668	mir-216	132	104	91	110	126
HeLa	328759	668	mir-216	797	40	82	203	223
T47D	328759	668	mir-216	123	86	87	122	223
T47Dp53	328759	668	mir-216	423	99	93	108	109
HeLa	327896	314	mir-22	95	103	94	106	144
HeLa	338836	660	mir-23a	303	97	96	121	114
HeLa	328084	743	mir-30a	286	89	92	153	125
HeLa	340350	855	mir-95 (Mourelatos)	132	101	94	112	177

When treatment of cells with oligomeric compounds resulted in greater than 750% cells in subG1 phase, these oligomeric compounds were deemed to be "hits," in that they appear to cause an increase in apoptosis, resulting in hypodiploid DNA contents. When treatment of cells with oligomeric compounds resulted in greater than 140% cells in G1-phase, these oligomeric compounds were deemed "hits," as they appeared to cause an arrest or delay in G1-phase and/or blocked entry into S-phase of the cell cycle. When treatment of cells with oligomeric compounds resulted in greater than 140% cells in S-phase, these oligomeric compounds were deemed "hits," as they appeared to cause an arrest or delay in DNA synthesis. When treatment of cells with oligomeric compounds resulted in greater than 140% cells in G2/M phase, these oligomeric compounds were deemed "hits," as they appeared to cause an arrest or delay in the transition into mitosis, and/or in cell division, itself.

From these data, it was observed that 48-hours after treatment of the various cell lines with the oligomeric compounds, ISIS Number 183891 targeting the kinesin-like 1/Eg5 mRNA results in a delay or arrest in G2/M phase of the cell cycle for all cell lines. Treatment of HeLa cells with ISIS Number 25691, targeted against the Drosha RNase III mRNA, resulted in an increased percentage of cells in S-phase as well as a significant percentage of cells in the subG1 and aneuploid categories, indicating that this oligomeric compound may interfere with DNA replication and/or maintenance of the integrity of the proper complement of genetic material.

In HeLa cells, ISIS 341787 (SEQ ID NO: 852) targeted against mir-125a (mouse), resulted in an arrest or delay in G2/M as well as an increased percentage of cells in the subG1 and

aneuploid categories, indicating that this oligomeric compound may interfere with cell division and equal chromosome segregation during mitosis.

In HeLa cells treated with ISIS 328744 (SEQ ID NO: 653) targeted against mir-182, an increase in the percentage of cells in the G2/M-phase of the cell cycle as well as in the subG1 category was observed, indicating that this oligomeric compound may interfere with cell division and equal chromosome segregation during mitosis. Notably, genetically normal cells (BJ and T47Dp53cells) were not affected by ISIS Number 328744, indicating that the oligomeric compound targeting miR-182 may selectively cause a cell cycle delay or arrest in cancer cells and not normal cells, and suggesting that this compound may be particularly useful as a therapeutic agent in the treatment of hyperproliferative disorders such as cancer.

In HeLa cells treated with ISIS 328759 (SEQ ID NO: 668) targeted against mir-216, a delay or arrest resulted in the G2/M-phase of the cell cycle was observed, as well as an increase in the percentage of cells in the subG1 and aneuploid categories, indicating that this oligomeric compound may interfere with cell division and equal chromosome segregation during mitosis.

Thus, it was observed that treatment of HeLa cells with oligomeric compounds targeting miRNAs is a effective means of identifying compounds that can block progression through various stages of the cell cycle. Notably, a transient increase in G1-phase was observed 24 hours after treatment of HeLa cells with oligomeric compounds targeting miRNAs; for example, oligomeric compounds ISIS Numbers 338769, 338836, 340350, and 338637 caused a transient increase in the percentage of cells delayed or arrested in G1-phase at the 24-hour time point, which, by the 48-hour time point, had shifted to a delay or arrest in S-phase. It was also noted that multiple oligomeric compounds targeting the same miRNA have the same effect on cell cycling. It was also observed that uniform 2'-MOE as well as 2'-MOE chimeric gapmer oligomeric compounds targeting the mature miRNA, as well as uniform 2'-MOE oligomeric compounds targeting the pri-miRNA often have the same effect.

Oligomeric compounds that delay, arrest or prevent cell cycle progression or induce apoptosis may be useful as therapeutic agents for the treatment of hyperproliferative disorders, such as cancer, cancer, as well as diseases associated with a hyperactivated immune response.

It is understood that BJ, B16, HeLa, A549, HMECs, T47D, T47Dp53, MCF7 or other cell lines can be treated with oligomeric compounds designed to mimic miRNAs in studies to examine their effects on progression through the cell cycle. Such oligomeric compounds are within the scope of the present invention.

Example 25: A bioinformatic approach to identification of miRNA targets

Several candidate RNA transcripts identified using the RACE-PCR methods described in Example 20 were the basis for a bioinformatic analysis of predicted targets bound to and/or potentially regulated by miRNAs. The complementarity between the miRNA used as a primer and the 3'-UTR of the RNA transcript identified by RACE-PCR was assessed using several methods. Transcripts identified by RACE-PCR were also analyzed using the FASTA sequence alignment program (accessible through the internet at, for example, www.ebi.ac.uk/fasta33) to find the best alignment between complementary sequences of the transcript and the miRNA used as a primer for RACE-PCR. When, using the default parameters, the FASTA alignment program resulted in the identification of the actual primer binding site (PBS) within the 3'-UTR of the RNA transcript as the sequence most complementary to the miRNA used as a primer in the RACE-PCR method, the candidate miRNA target transcript was specified by a plus sign (for example, see the "mir-143/PBS complementary?" column in Table 36 below). When the FASTA program failed to align the actual PBS with the sequence most complementary to the miRNA used in the RACE-PCR, the candidate miRNA target transcript was specified by a minus sign. When the FASTA program could be made to align with the sequence most complementary to the miRNA used in the RACE-PCR by decreasing the stringency of the FASTA program parameters, the candidate miRNA target transcript was specified by "±".

A global alignment was also performed to assess whether the sequence of the PBS within the RNA transcript identified by RACE-PCR was conserved between human and mouse orthologs of the RNA target. For example, in Table 36, below, strong conservation of PBS in the human and murine orthologs (homology from 80-100%) was indicated by a plus sign; moderate conservation (homology between 70-80%) was indicated by "±", and a minus sign indicates homology below 70%.

A variety of algorithms can be used to predict RNA secondary structures based on thermodynamic parameters and energy calculations. For example, secondary structure prediction can be performed using either M-fold or RNA Structure 2.52. M-fold can be accessed through the Internet at, for example, www.ibc.wustl.edu/-zuker/ma/form2.cgi or can be downloaded for local use on UNIX platforms. M-fold is also available as a part of GCG package. RNA Structure 2.52 is a windows adaptation of the M-fold algorithm and can be accessed through the Internet at, for example, 128.151.176.70/RNAstructure.html. The RNA Structure 2.52 program was used to analyze a series of 30-base fragments spanning the entire length of the human RNA transcript and their potential to hybridize with the miRNA used as a primer in the RACE-PCR, allowing the prediction of the lowest absolute free energy peak representing the most likely site of hybridization (including bulged regions) between the

miRNA and the RNA target. If the free energy peak representing the hybridization between the miRNA and the PBS of the RNA transcript identified by RACE-PCR was among the top five peaks predicted by the RNA Structure 2.52 program, the transcript was given a plus sign, "+". If the free energy peak representing the hybridization between the miRNA and the PBS was in the top five to ten peaks predicted by RNA Structure 2.52, the transcript was given a plus/minus sign, "±", and if the peak representing the hybridization between the miRNA and the PBS was below the top ten peaks predicted by RNA Structure 2.52, the transcript was given a minus sign, "-".

A list of the RNA transcript targets identified by RACE-PCR employing the mir-143 miRNA as a specific primer is shown in Table 36.

Table 36

Potential RNA targets of the mir-143 miRNA

RNA transcript target	SEQ ID NO:	PBS conserved?	RNA Structure peak?	mir-143/PBS complementary ?
Matrix metalloproteinase 2	819	+	-	+
Sec24	829	-	+/-	+
Tripartite motif-containing 32	828	+/-	+	+/-
RAN	824	+/-	+	+
Cystatin B	802	-	+	+
Glucocorticoid induced transcript 1	839	+	+/-	+
Protein phosphatase 2	809	+	+	+
Polycystic kidney disease 2	822	-	-	-
Mannose-6-phosphate receptor	801	+/-	-	+
Mitotic control protein dis3 homolog	817	+	+	-
Chromosome 14 ORF 103	813	+	+/-	-
Rho GDP dissociation inhibitor beta	823	-	-	-
Glyoxalase I	816	+	+	+
Zinc finger protein 36, C3H type-like 1	818	+	+/-	+
LIM domain only 4	804	+	+	+

Note that four genes (Sec24, cystatin B, polycystic kidney disease 2, and Rho GDP dissociation inhibitor beta) did not have murine orthologs to compare in a global analysis of the PBS. Because these RNA transcripts were identified as being bound by the mir-143 miRNA used as a primer in the RACE-PCR approach previously described, the mir-143 miRNA is predicted to serve a regulatory role in expression or activity of one or more or all of these RNA transcripts. Of particular note are three targets, protein phosphatase 2, glyoxalase I, and LIM domain only 4 (LMO4) mRNAs, for which all three analyses yielded a positive result. That all three parameters

assessed yielded a positive result suggests that these mRNAs are probable targets of mir-143.

The well-studied *C. elegans* lin-4 miRNA interaction with its lin-28 mRNA target was used as the starting point for a bioinformatics approach to the identification of miRNA binding sites in target nucleic acids. Lin-4 has been experimentally determined to bind at a single site on the lin-28 mRNA. Herein, as a primary determinant of miRNA-target interactions, it was hypothesized that the bimolecular hybridization free energies ($\Delta G°_{37}$) of the interaction of the miRNA with a true target site would be more negative than the $\Delta G°_{37}$ of other interactions of the miRNA with other binding sites. The nucleotide sequence of the lin-28 mRNA was assessed by computationally deriving 30-nucleotide windows, starting with the first nucleotide of the sequence and defining the first nucleotide in each window by shifting 1 nucleotide in the 3' direction. Each window was assessed by hybridizing the 30-nucleotide sequence in the window with the lin-4 miRNA and disallowing unimolecular interactions, thereby spanning the entire length of the lin-28 mRNA, and the resulting $\Delta G°_{37}$ value was plotted against the start position of the window. It was observed that the bimolecular hybridization between the true lin-4 binding site and the lin-28 mRNA had the lowest $\Delta G°_{37}$ value, supporting our hypothesis and our bioinformatic approach to the identification of miRNA binding sites in target nucleic acids.

The mitogen-activated protein kinase 7/extracellular signal-regulated kinase 5 (ERK5) (GenBank Accession NM_139032.1, incorporated herein as SEQ ID NO: 861) mRNA transcript was previously computationally predicted to be regulated by mir-143 miRNA binding in the 3'-UTR regions (Lewis et al., *Cell,* **2003**, *115*, 787-798). In order to identify mir-143 binding sites in the ERK5 mRNA, a bimolecular hybridization free energy assessment was performed by performing a hybridization walk to assess possible mir-143 binding sites along the entire length of the ERK5 mRNA. A strong negative $\Delta G°_{37}$ value (-20.1) was found at the previously predicted mir-143 binding site in the 3'-UTR, lending further support to our method. Surprisingly, two additional, and novel, mir-143 binding sites with more negative $\Delta G°_{37}$ values, as well as a third mir-143 binding site with a comparable $\Delta G°_{37}$ value were also identified. Using the ERK5 sequence (GenBank Accession NM_139032.1) as a reference, these binding sites encompass nucleotides 937-966 with a $\Delta G°_{37}$ value of (-22.8), nucleotides 2041-2070with a $\Delta G°_{37}$ value of (-20.6) and nucleotides 2163-2192 with a $\Delta G°_{37}$ value of (-19.3). See Figure 1. Thus, three novel mir-143 binding sites (and, thus, a potential regulatory sites) were identified within the coding sequence of the ERK5 gene. Thus, this method of screening for miRNA binding sites by a bimolecular hybridization free energy assessment can be used to confirm previously predicted sites, and further allows the identification of novel miRNA target nucleic

acid binding sites. It is believed that this method may more closely mimic the energetic mechanism by which a miRNA scans a target nucleic acid to find its interaction site. In subsequent experiments, the predicted mir-143 binding sites within the ERK5 coding sequence were also tested using the reporter system described below.

Example 26: Northern analysis of miRNA expression

As described in the adipocyte differentiation assay, the oligomeric compounds ISIS Number 327889 (SEQ ID NO: 307), targeted to mir-23b, and ISIS Number 327876 (SEQ ID NO: 294), targeted to mir-29b-1, were found to reduce the expression of several hallmark genes of adipocyte differentiation, indicating that mir-23b and mir-29b-1 may play a role in adipocyte differentiation, and that oligomeric compounds targeting these miRNAs may be useful as agents blocking cellular differentiation. Therefore, the expression of mir-23b and mir-29b was assessed by Northern blot of total RNA from multiple tissues. To detect the mir-23b and mir-29b-1 targets, target specific DNA oligonucleotide probes with the sequences GTGGTAATCCCTGGCAATGTGAT (SEQ ID NO: 307) and AACACTGATTTCAAA TGGTGCTA (SEQ ID NO: 294), respectively, were synthesized by IDT (Coralville, IA). The oligo probes were 5' end-labeled with T4 polynucleotide kinase with (γ-^{32}P) ATP (Promega). To normalize for variations in loading and transfer efficiency membranes are stripped and probed for U6 RNA. Total RNA from mouse and human tissues as well as total RNA from human adipocytes and HepG2 cells was probed in Northern blot analyses, using methods described in Example 14.

By Northern analyses, the mir-23b miRNA was found to be most highly expressed in human kidney tissue as well as in adipose tissue from ob/ob mice, and was also highly expressed in human liver, adipocytes, preadipocytes and HepG2 cells. Moderate expression of mir-23b was also noted in murine kidney tissue. The mir-29b-1 miRNA was found to be most highly expressed in human and mouse kidney, and was also expressed in human liver, adipocytes, preadipocytes, and HepG2 cells, as well as in murine adipose tissue and liver. Levels of both the mir-23b and mir-29b-1 miRNAs were also found to be upregulated in human differentiated adipocytes.

Similarly, target specific DNA oligonucleotide probes for mir-16, mir-15a, and let-7a were designed and used in Northern blot analyses to assess expression of these miRNAs in human and mouse tissues. The mir-16 and mir-15a miRNAs were each found to be most highly expressed in human spleen, heart, testes, and kidney, and expression was also observed in liver as well as HEK293 and T47D cells. Additionally, low levels of expression of the mir-16 miRNA

were observed in NT2 cells. The let-7a miRNA was most highly expressed in human and murine kidney, and expression was also observed in human and murine liver. Additionally, low levels of let-7a expression were found in HepG2 cells.

To detect the mir-21 miRNA in Northern blot analyses, a target specific DNA oligonucleotide probe with the sequences TCAACATCAGTCTGATAAGCTA (SEQ ID NO: 335) was synthesized by IDT (Coralville, IA). The oligo probes were 5' end-labeled with T4 polynucleotide kinase with (γ-^{32}P) ATP (Promega). Twenty micrograms of total RNA from human Promyelocytic Leukemia HL-60 cells, A549, HeLa, HEK293, T47D, HepG2, T-24, MCF7, and Jurkat cells was was fractionated by electrophoresis through 15% acrylamide urea gels using a TBE buffer system (Invitrogen). RNA was transferred from the gel to HYBOND™-N+ nylon membranes (Amersham Pharmacia Biotech, Piscataway, NJ) by electroblotting in an Xcell SureLock™ Minicell (Invitrogen). Membranes were fixed by UV cross-linking using a STRATALINKER™ UV Crosslinker 2400 (Stratagene, Inc, La Jolla, CA) and then probed using Rapid Hyb buffer solution (Amersham) using manufacturer's recommendations for oligonucleotide probes. To normalize for variations in loading and transfer efficiency membranes are stripped and probed for U6 RNA. High levels of expression of mir-21 were observed in A549 and HeLa cells; in fact, levels of mir-21 expression were noted to be among the highest of any of the miRNAs observed in HeLa cells.

Example 27: Reporter systems for assaying activity of oligomeric compounds targeting or mimicking miRNAs

Reporter systems have been developed herein to assess the ability of miRNA mimics to provoke a gene silencing response and to assess whether antisense oligomeric compounds targeting miRNAs can inhibit gene silencing activity. The T-REx™-HeLa cell line (Invitrogen Corp., Carlsbad, CA) was used for either stable or transient transfections with plasmids constitutively expressing miRNAs, pre-miRNAs, pri-miRNAs or mimics thereof, and, in some cases, antisense oligomeric compounds targeting the expressed miRNA were also transfected into the cells. It is understood that other mammalian cells lines can also be used in this reporter system. T-REx™-HeLa cells were routinely cultured in DMEM, high glucose (Invitrogen Corporation, Carlsbad, CA), supplemented with 10% fetal bovine serum (Invitrogen Corporation). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells were harvested when they reached 90% confluence, and on the day before transfection with expression or reporter plasmids (described in detail below), the T-REx™-HeLa cells were seeded onto 24-well plates at 50,000 cells/well. The following day, cells

were transfected according to standard published procedures with various combinations of plasmids using 2 μg Lipofectamine™ 2000 Reagent (Invitrogen) per μg of plasmid DNA. When transfecting oligomeric compounds, 1-3 μg of Lipofectamine™ 2000 Reagent was used per 100 nM oligomeric compound.

Plasmids used are as follows: The pcDNA3.1©/NT-GFP (Invitrogen) plasmid, containing a CMV promoter controlling expression of a GFP reporter sequence at the N-terminus of the transcription start site was used as a control plasmid. The pcDNA3.1©/NT-GFP-mir-143 sensor plasmid contains (in addition to the elements above) three 22-nucleotide sites encoding the mir-143 miRNA binding site, downstream of the GFP coding sequence and upstream of the polyadenylation signal. The pCR3-pri-mir-143 plasmid ("pri-mir-143") is a CMV promoter-driven constitutive expression plasmid which expresses the 110-nucleotide mir-143 pri-miRNA sequence (SEQ ID NO: 38), to act as a mir-143 pri-miRNA mimic. The pCR3-pri-mir control ("pri-mir-control") is a CMV promotor-driven constitutive expression plasmid which is designed to express a similar 110-nucleotide pri-miRNA sequence (AGCAGCGCAGCGCCCTGTCTCCCAGCCAAGGTGGAACCTTCTGGGA AGCGGTCAGTTGGGAGTCCCTTCCCTGAAGGTTCCTCCTTGGAAGAGAGAAGTTGTT CTGCAGC; SEQ ID NO: 862) wherein the mature mir-143 sequence has been replaced with an unrelated sequence and the predicted complementary strand opposite it within the pri-miRNA structure is replaced with a nearly complementary sequence in order to preserve the stem loop as well as the bulge structure of the natural mir-143 pri-miRNA. Additionally, in order to test the effect of an oligomeric compound targeting a miRNA, the T-REx™-HeLa cells were also transfected with the uniform 2'-MOE phosphorothioate (PS) antisense oligomeric compound ISIS Number 327901 (SEQ ID NO: 319), targeted to mir-143 previously described.

Twenty-four hours post-transfection, cells were trypsinized and GFP fluorescence was analyzed by flow cytometry. Results are shown in Table 37.

Table 37
Mean GFP fluorescence after transfection of T-REx™-HeLa cells

Treatment					Mean fluorescence
pri-mir control	pri-mir-143	GFP control	GFP mir-143 sensor	327901 oligo	
-	-	-	-	-	2.2
+	-	-	-	-	2.7
-	+	-	-	-	2.6
-	-	+	-	-	7.9
+	-	+	-	-	22.7
-	+	+	-	-	9.6
-	-	-	+	-	12.4
+	-	-	+	-	21.8

-	+	-	+	-	5.3
-	+	-	+	+	4.1
-	-	-	+	+	4.2
-	+	-	-	+	3.7

Plus signs, "+", indicate the presence of the expression plasmid or oligomeric construct in transfectants; minus signs "-", indicate the absence of same. Mean fluorescence is measured in arbitrary units.

In cells transfected with the sensor plasmid and expressing the mir-143 pri-miRNA mimic from the pCR3-pri-mir-143 plasmid, the mir-143 miRNA is expected to be processed endogenously, allowing it to bind as a mature miRNA to the RNA transcript encoding GFP and containing the mir-143 binding sites expressed from the reporter plasmid, resulting in cleavage of the reporter transcript and a decrease in fluorescence as compared to the control plasmid. From the data shown in Table 37, it was observed that expression of the pCR3-pri-mir-143 plasmid results in an inhibition of expression of GFP indicated by a decrease in fluorescence produced by the pcDNA3.1©/NT-GFP-mir-143 sensor plasmid, whereas expression of the pCR3-pri-mir control plasmid had no effect on GFP reporter expression. Neither the pCR3-pri-mir control nor the pCR3-pri-mir-143 plasmid had any inhibitory effect on GFP expression from the pcDNA3.1©/NT-GFP control plasmid. Thus, the mir-143 pri-miRNA mimic oligomeric compound silences the expression of RNA transcribed from a reporter plasmid containing mir-143 target sites.

In a further study, T-REx™-HeLa cells transfected with the pcDNA3.1©/NT-GFP-mir-143 sensor plasmid were treated at various dosages with the following oligomeric compounds: 1) a double-stranded RNA oligomeric compound ("ds-mir-143") composed of ISIS Number 342199 (TGAGATGAAGCACTGTAGCTCA; SEQ ID NO: 220) representing the mir-143 sense sequence, hybridized to its perfect complement ISIS Number 342200 (TGAGCTACAGTGCTTCATCTCA; SEQ ID NO: 319); 2) a negative control dsRNA ("ds-Control"), representing a 10-base mismatched sequence antisense to the unrelated PTP1B mRNA, composed of ISIS Number 342427 (CCTTCCCTGAAGGTTCCTCC; SEQ ID NO: 863) hybridized to its perfect complement ISIS Number 342430 (GGAGGAACCTTCAG GGAAGG; SEQ ID NO: 864); 3) the pCR3-pri-mir-143 expression plasmid ("pCR3-pri-mir-143") which expresses the 110-nucleotide mir-143 pri-miRNA; 4) the pCR3-pri-mir control ("pri-mir-control"); 5) an *in vitro* transcribed RNA oligomeric compound ("hairpin mir-143") representing the 110bp fragment of the mir-143 pri-miRNA molecule (SEQ ID NO: 38) plus an additional two cytosine nucleobases from the T7 promoter at the 5' end; and 6) an *in vitro*

transcribed RNA oligomeric compound ("hairpin control") (SEQ ID NO: 862) representing a similar hairpin structure except that the mature mir-143 sequence and its complementary sequence within the pri-miRNA hairpin structure were replaced with sequences unrelated to mir-143. The RNA hairpin oligomeric compounds were *in vitro* transcribed using the MAXIscript Kit (Ambion Inc., Austin, TX) according to the manufacturer's protocol, beginning with a DNA template amplified by PCR. GFP fluorescence of treated cells was assessed using the methods described above, and it was observed that the ds-mir-143 oligomeric compound mimic inhibited expression of GFP from the sensor plasmid in a dose dependent manner. In a further embodiment, pcDNA3.1©/NT-GFP-mir-143 sensor-expressing cells treated with 20 nM mir-143 dsRNA oligomeric compound were additionally treated with 4-, 20- or 100 nM uniform 2'-MOE oligomeric compound ISIS Number 327901 (SEQ ID NO: 319), or 4-, 20- or 100 nM uniform 2'-MOE scrambled mir-143 control ISIS Number 342673 (SEQ ID NO: 758) to assess the ability of compounds to inhibit the inhibitory effect of the mir-143 dsRNA mimic. At all three concentrations, the oligomeric compound ISIS Number 327901 targeting mir-143 blocked the inhibitory effect of the mir-143 dsRNA oligomeric compound, exhibited as a recovery of GFP fluorescence.

In one embodiment, an expression system based on the pGL3-Control (Promega Corp., Madison WI) vector containing a CMV promoter controlling expression of a luciferase reporter sequence was used in transient transfections of HeLa cells with plasmids expressing miRNA or pri-miRNA mimics. To assess the ability of miRNA mimics to bind and regulate the expression of the luciferase reporter gene, two reporter plasmids were constructed: 1) a synthetic DNA fragment comprising two sites perfectly complementary to mir-143 were inserted into the pGL3-Control luciferase reporter vector, to create the pGL3-mir-143 sensor plasmid, and 2) a DNA fragment comprising the 3'-UTR of the LIM domain only 4 (LMO4) gene (predicted to be regulated by mir-143) was inserted into pGL3-Control to create pGL3-LMO4; this fragment was PCR-amplified using a primer beginning at position 1261 of the LMO4 sequence (GenBank Accession NM_006769.2, incorporated herein as SEQ ID: 809) and the downstream primer hybridizing to the poly-A tail. In each of these plasmids, the target site was placed downstream of the luciferase coding sequence and upstream of the polyadenylation signal in the 3'-UTR of the luciferase reporter vector. The unmodified pGL3-Control luciferase reporter vector was used as a control.

HeLa cells were routinely cultured and passaged as described, and on the day before transfection with expression or reporter plasmids, the HeLa cells were seeded onto 24-well plates 50,000 cells/well. Cells were transfected according to standard published procedures with

various combinations of plasmids using 2 μg Lipofectamine™ 2000 Reagent (Invitrogen) per μg of plasmid DNA, or, when transfecting oligomeric compounds, 1.25 μg of Lipofectamine™ 2000 Reagent per 100 nM oligonucleotide or double-stranded RNA. The luciferase signal in each well was normalized to the *Renilla* luciferase (RL) activity produced from a co-transfected plasmid, pRL-CMV, which was transfected at 0.5 μg per well. Cells were treated at various dosages (4 nM, 20 nM, and 100 nM) with the following oligomeric compound mimics: 1) "ds-mir-143," 2) "ds-Control," 3) "pCR3-pri-mir-143," or 4) "pri-mir-control," as described *supra*. In accordance with methods described in Example 12, *supra*, a luciferase assay was performed 48-hours after transfection. Briefly, cells were lysed in passive lysis buffer (PLB; Promega), and 20 ul of the lysate was then assayed for RL activity using a Dual Luciferase Assay kit (Promega) according to the manufacturer's protocol. The results below are an average of three trials and are presented as percent pGL3-Control luciferase expression normalized to pRL-CMV expression (RL). The data are shown in Table 38.

Table 38

Luciferase assays showing effects of oligomeric compounds mimicking mir-143

	luciferase expression (% lucif.only control)		
treatment	pGL3-Control	pGL3-mir-143 sensor	pGL3-LMO4
no luciferase (pRL)	0.3	0.3	0.4
luciferase (pRL) only	100.0	101.0	100.0
ds-mir-143 (4 nM)	101.5	14.5	151.6
ds-mir-143 (20 nM)	123.8	8.0	140.1
ds-mir-143 (100 nM)	131.8	7.1	128.4
ds-Control (4 nM)	133.6	144.5	172.4
ds-Control (20 nM)	126.1	169.8	151.6
ds-Control (100 nM)	123.0	151.3	151.5
pCR3-pri-mir-143 (0.25ug)	75.6	58.6	101.9
pCR3-pri-mir-143 precursor (0.5ug)	76.6	50.7	95.7
pCR3-pri-mir-143 (1 ug)	64.7	35.0	82.5
pri-mir control (0.25 ug)	90.3	78.3	114.8
pri-mir control (0.5 ug)	57.3	61.8	95.4
pri-mir control (1 ug)	67.9	64.9	74.8

From these data, it was observed that the mir-143 dsRNA oligomeric compound and the mir-143 pri-miRNA mimic expressed from the pCR3-pri-mir-143 expression plasmid both inhibited luciferase activity from the pGL3-mir-143 sensor plasmid in a dose-dependent manner.

In another embodiment, HeLa cells were transfected with 0.03 μg pGL3-mir-143 sensor plasmid and 0.01 μg pRL-CMV plasmid, and, in addition, (except those samples described below as "without mir-143 pri-miRNA,") were also transfected with 0.01 μg of an expression plasmid designed to express a mir-143 pri-miRNA mimic comprising a larger 430-nt fragment of

the mir-143 primary miRNA transcript, referred to as "pCR3-pri-mir-143 (430)" (AGGTTTGGTCCTGGGTGCTCAAATGGCAGGCCACAGACAGGAAACACAG TTGTGAGGAATTACAACAGCCTCCCGGCCAGAGCTGGAGAGGTGGAGCCCAGGTCC CCTCTAACACCCCTTCTCCTGGCCAGGTTGGAGTCCCGCCACAGGCCACCAGAGCGG AGCAGCGCAGCGCCCTGTCTCCCAGCCTGAGGTGCAGTGCTGCATCTCTGGTCAGTT GGGAGTCTGAGATGAAGCACTGTAGCTCAGGAAGAGAGAAGTTGTTCTGCAGCCAT CAGCCTGGAAGTGGTAAGTGCTGGGGGGTTGTGGGGGGCCATAACAGGAAGGACA GAGTGTTTCCAGACTCCATACTATCAGCCACTTGTGATGCTGGGGAAGTTCCTCTAC ACAAGTTCCCCTGGTGCCACGATCTGCTTCACGAGTCTGGGCA; SEQ ID NO: 871). It was observed that the mir-143 pri-miRNA mimic expressed by pCR3-pri-mir-143 (430) inhibits luciferase expression from the pGL3-mir-143 sensor plasmid. To further evaluate the ability of the mir-143 pri-miRNA mimic to inhibit luciferase activity from the sensor plasmid, and to assess the ability of oligomeric compounds to interfere with the inhibition of pGL3-mir-143 sensor luciferase expression by the mir-143 pri-miRNA mimic, pGL3-mir-143 sensor-expressing HeLa cells treated with pCR3-pri-mir-143 (430) were additionally treated with varying concentrations (0-, 6.7- or 20 nM) of the following oligomeric compounds: 1) ISIS Number 327901 (SEQ ID NO: 319), a uniform 2'-MOE oligomeric compound targeting mir-143; 2) ISIS Number 342673 (SEQ ID NO: 758), a uniform 2'-MOE scrambled control; or 3) ISIS Number 327924 (SEQ ID NO: 342) targeting an unrelated miRNA (mir-129-2). ISIS Numbers 342673 and 327924 were used as negative controls. HeLa cells transfected with the pRL-CMV and pGL3-mir-143 sensor plasmids, but not treated with the pCR3-pri-mir-143 (430) hairpin precursor served as a control. In this experiment, the luciferase assay was performed 24-hours after transfection. The data are presented in Table 39 as relative luciferase activity (normalized to RL expression levels). Where present, "N.D." indicates "no data."

Table 39
Effects of oligomeric compounds on mir-143 pri-miRNA mimic-mediated inhibition of luciferase expression

Treatment	SEQ ID NO	Relative luciferase activity		
		Dose of oligomeric compound		
		0 nM	6.7 nM	20 nM
327901	319	0.97	4.0	6.4
342673 negative control	758	0.97	1.3	1.5
327924 negative control	342	0.97	0.8	1.2
without pCR3-pri-mir-143(430)	N/A	13.8	N.D.	N.D.

From these data, it was observed that the oligomeric compound ISIS Number 327901 targeting mir-143 blocked the inhibitory effect of the mir-143 pri-miRNA mimic, exhibited as a 4- to 6.4-fold recovery of luciferase activity in HeLa cells expressing the pGL3-mir-143 sensor plasmid.

More than four-hundred target genes have been predicted to be regulated by miRNA binding to the 3'-UTR regions of the mRNA transcript (Lewis et al., *Cell,* **2003**, *115*, 787-798). For example, at least six genes have been reported to bear regulatory sequences in their 3'-UTRs which are predicted to be bound by the mir-143 miRNA; these include the inwardly rectifying potassium channel Kir2.2 (GenBank Accession AB074970, incorporated herein as SEQ ID NO: 872), synaptotagmin III (GenBank Accession BC028379, incorporated herein as SEQ ID NO: 873), mitogen-activated protein kinase 7/extracellular signal-regulated kinase 5 (ERK5) (GenBank Accession NM_139032.1, SEQ ID NO: 861), protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform (PPP2CB) (GenBank Accession NM_004156.1, SEQ ID NO: 814), glyoxalase I (GLO1) (GenBank Accession NM_006708.1, SEQ ID NO: 821), and LIM domain only 4 (LMO4) (GenBank Accession NM_006769.2, SEQ ID NO: 809). It should be noted that one third of miRNA targets predicted in the study by Lewis, et al. are expected to be false positives (Lewis et al., *Cell,* **2003**, *115*, 787-798).

Because the present inventors independently identified the PPP2CB and GLO1 genes as potential targets of mir-143 by the RACE-PCR methods as described in Example 20, these targets were selected for further study. In addition, and described in Example 25, a novel mir-143 binding site (and, thus, a potential regulatory site) was identified within the coding sequence of the ERK5 gene; this predicted mir-143 binding site within the ERK5 coding sequence was also tested in these reporter systems.

In some embodiments, an expression system based on the pGL3-Control (Promega Corp., Madison WI) reporter vector and comprising predicted miRNA binding sites was used in stable transfections of HeLa cells, selecting for cells that have integrated the reporter plasmid into their genome. Because pGL3-based reporter vectors have no selectable marker for antibiotic resistance, a neomycin-resistance (Genetecin) gene was cloned into the pCR2 plasmid (Invitrogen Life Technologies, Carlsbad, CA) to create the pCR2-neo plasmid, and pCR2-neo was co-transfected into HeLa cells with the pGL3-mir-143-sensor plasmid at a ratio of one pCR2-neo plasmid to ten pGL3-mir-143-sensor plasmids. Co-transfected cells were then selected for the presence of the Genetecin marker and assayed for luciferase activity; Genetecin-resistant cells are very likely to have also integrated the luciferase reporter into their genome.

Establishment of stably-transfected cells:

One day prior to transfection, approximately 750,000 HeLa cells are seeded onto a 10cm dish or T-75 flask and grown in complete medium overnight at 37° C. The next day, 10 μg of pGL3-mir-143-sensor plasmid and 1 μg pCR2-neo are mixed in 2 ml OPTI-MEM™ (Invitrogen Corporation, Carlsbad, CA). (Linearization of circular plasmids by digestion with restriction enzyme may increase the number of stable transfectants per μg transforming DNA, but is not an essential step). 10 μl LIPOFECTIN™ reagent (Invitrogen Corporation, Carlsbad, CA) is mixed with 2 ml OPTI-MEM™. The plasmid/OPTI-MEM™ and OPTI-MEM™/LIPOFECTIN™ mixtures are then mixed together, and an additional 11ml OPTI-MEM™ is added, and the resulting 15 ml cocktail is added to the cells. Cells are incubated in the plasmid/OPTI-MEM™/LIPOFECTIN™ cocktail for approximately 4 hours at 37° C, after which the cocktail is removed and replaced with fresh complete medium. The following day, cells are trypsinized and transferred to a T-175 flask. Media containing the selection agent, 500μg/ml G418 (Geneticin; GIBCO/Life Technologies, Gaithersburg, MD), is added and cells are grown at 37° C. Cells are re-fed daily with fresh media containing the selection agent until the majority of the cells appear to have died off and isolated colonies of neomycin-resistant cells appear. In cases where subcloning is desired, selected neomycin-resistant cells are trypsinized and plated at a concentration of 0.5 cells/well in 96-well plates, maintaining the cells in 500μg/ml G418 selection media.

In one embodiment, five stably-transfected, neomycin-resistant, luciferase-positive, pGL3-mir-143-sensor cell clones were isolated, subcloned and selected for further testing with oligomeric compounds of the present invention. Cells stably expressing the luciferase reporter and comprising one or more miRNA binding sites were then transfected with oligomeric compounds mimicking miRNAs, pre-miRNAs or pri-miRNAs in order to assess the ability of these miRNA mimics to bind and regulate the expression of the luciferase reporter.

An expression system based on the pGL3-Control (Promega Corp., Madison WI) reporter vector and comprising predicted miRNA binding sites was used in transient transfections of HeLa cells with plasmids expressing oligomeric compounds mimicking miRNAs, pre-miRNAs or pri-miRNAs in order to assess the ability of these miRNA mimics to bind and regulate the expression of the luciferase reporter. The effect of increasing the copy number of the miRNA-binding site in the target was also tested by including multiple binding sites in artificial reporter constructs. It is understood that the presence of multiple miRNA-binding sites in a target can include binding sites for different miRNAs.

The following reporter plasmids were constructed by cloning the specified fragment

into the XbaI site of the pGL3-control plasmid, placing the potential miRNA-binding site in the 3'-UTR of the luciferase reporter: The reporter plasmid pGL3-bulge(x3) contains three contiguous copies of the sequence (TGAGCTACAGCTTCATCTCA; herein incorporated as SEQ ID NO: 874) which represents a sequence complementary to the mir-143 miRNA except that it is missing 2 nucleotides such that the mir-143 miRNA is presumed to adopt a bulged structure when it hybridizes to this target sequence. The pGL3-GLO1 reporter plasmid contains a DNA fragment comprising the 3'-UTR of the GLO1 sequence; this fragment was PCR-amplified using a primer beginning at nucleotide number 621 of the GLO1 sequence (GenBank Accession NM_006708.1, SEQ ID NO: 821) and the downstream primer hybridizing to the poly A tail. The pGL3-PP2A reporter plasmid contains a DNA fragment comprising the 3'-UTR of the PP2A gene; this fragment was PCR-amplified using a primer beginning at nucleotide number 921 of the PP2A sequence (GenBank Accession NM_004156.1) and the downstream primer hybridizing to the poly A tail. The reporter plasmid pGL3-ERK5-3'-UTR(x1) contains one copy of the sequence TATTCTGCAGGTTCATCTCAG (herein incorporated as SEQ ID NO: 875), found in the 3'-UTR of ERK5 and predicted by Lewis, et al. to be bound by the mir-143 miRNA, and the reporter plasmid pGL3-ERK5-3'UTR(x3) has three contiguous copies of this sequence. The reporter plasmid pGL3-ERK5-3'UTR(ext) contains one copy of the sequence CGGCTTGGATTATTCTGCAGGTTCATCTCAGACCCACCTTT (herein incorporated as SEQ ID NO: 876), which includes an additional ten nucleotides at either end of the mir-143 binding site in 3'-UTR of ERK5 predicted by Lewis, et al. (Lewis et al., *Cell,* **2003**, *115*, 787-798). The plasmids pGL3-ERK5-cds(x1), pGL3-ERK5-cds(x2), pGL3-ERK5-cds(x3), and pGL3-ERK5-cds(x5) contain one, two, three or five contiguous copies, respectively, of the novel mir-143 binding site (TTGAGCCCAGCGCTCGCATCTCA; herein incorporated as SEQ ID NO: 877) we identified within the coding sequence of ERK5. The unmodified pGL3-Control luciferase reporter vector was used as a negative control, and the pGL3-mir-143 sensor reporter plasmid was used as a positive control.

HeLa cells were routinely cultured and passaged as described. In some embodiments, HeLa cells were transfected with 0.05 μg of the relevant pGL3-sensor plasmid and 0.01 μg pRL-CMV plasmid. Additionally, in some embodiments, cells were treated at various dosages (11 nM, 33 nM, and 100 nM) with the following oligomeric compound mimics: 1) ds-mir-143, or 2) ds-Control as described. In accordance with methods described in Example 12, a luciferase assay was performed 24-hours after transfection. The results, shown in Tables 40 and 41, are an average of three trials. Data are presented as percent untreated control (luciferase plasmid only, not treated with oligomeric compound) luciferase expression, normalized to pRL-CMV levels.

Table 40

Effects of oligomeric compounds mimicking mir-143 on luciferase expression

Reporter plasmid	ds-mir-143			ds-control		
	11 nM	33 nM	100 nM	11 nM	33 nM	100 nM
pGL3-Control	90.7	94.2	72.5	113.4	79.6	87.0
pGL3-bulge(x3)	50.7	35.4	17.2	111.3	82.6	84.7
pGL3-ERK5-3'UTR(x1)	81.9	84.7	62.2	103.2	79.6	77.6

From these data, it was observed that, while treatment of HeLa cells expressing the reporter plasmids with the ds-control did not appear to significantly affect luciferase expression, the mir-143 dsRNA mimic compound inhibited luciferase activity from the pGL3-bulge(x3) sensor plasmid in a dose-dependent manner.

Table 41

Effects of oligomeric compounds mimicking mir-143 on luciferase expression

Reporter plasmid	ds-mir-143			ds-control		
	11 nM	33 nM	100 nM	11 nM	33 nM	100 nM
pGL3-Control	110.2	124.3	92.3	114.1	95.6	103.0
pGL3-mir-143 sensor	15.0	15.0	11.1	114.5	108.9	97.1
pGL3-bulge(x3)	36.1	33.9	22.2	109.5	103.2	92.4
pGL3-ERK5-3'UTR(x1)	92.2	108.1	81.9	106.2	99.6	90.1
pGL3-ERK5-3'UTR(x3)	51.7	51.0	28.2	104.6	103.4	95.7
pGL3-ERK5-cds(x1)	101.3	115.4	77.4	100.6	102.1	96.2
pGL3-ERK5-cds(x2)	92.7	113.8	63.6	111.3	99.2	90.4
pGL3-ERK5-cds(x3)	73.5	77.9	49.4	105.2	96.6	79.9
pGL3-ERK5-cds(x5)	49.4	44.5	23.9	103.0	113.4	89.9
pGL3-ERK5-3'UTR(ext)	89.0	106.7	81.4	96.8	108.9	89.4

From these data it was observed that treatment of HeLa cells expressing the pGL3-bulge(x3) reporter plasmid with the ds-mir-143 miRNA mimic oligomeric compound resulted in a dose-dependent inhibition of luciferase activity while the ds-control oligomeric compound had no effect as described previously. Treatment of HeLa cells expressing the pGL3-ERK5-3'UTR(x1) (containing one copy of the mir-143 binding site predicted by Lewis, et al.) with the ds-mir-143 mimic oligomeric compound did not inhibit luciferase activity, although increasing the number of potential mir-143 binding sites in the pGL3-ERK5-3'UTR(x3) reporter plasmid to three appeared to favor the binding of the ds-mir-143 mimic and inhibition of luciferase activity. Treatment of cells expressing the pGL3-ERK5-cds(x1) or pGL3-ERK5-cds(x2) reporter plasmids bearing a one or two copies, respectively, of the novel mir-143 binding site identified in the coding sequence of the ERK5 gene with 11- or 33 nM of the ds-mir-143 mimic oligomeric compound did not appear to inhibit luciferase activity, although treatment with 100 nM of the ds-mir-143 mimic did reduce luciferase expression. Treatment of cells expressing the pGL3-ERK5-

cds(x3) or pGL3-ERK5-cds(x5) reporter plasmids, bearing three or five of copies, respectively, of the novel mir-143 binding site in the ERK5 coding sequence, with the ds-mir-143 mimic oligomeric compound resulted in a reduction in luciferase activity. The pGL3-ERK5-cds(x5) reporter plasmid exhibited a dose-responsiveness with increasing concentration of the mir-143 mimic oligomeric compound. Taken together, these results support the conclusion that multiple miRNAs and miRNA binding sites may cooperate to silence gene expression.

In order to assess the ability of miRNAs to bind predicted miRNA binding sites and regulate the expression of the luciferase reporter, in some embodiments, expression systems based on the pGL3-Control (Promega Corp., Madison WI) reporter vector and comprising either a mir-15a, mir-21, or a mir-23b miRNA binding site were developed and used in transient transfections of HeLa cells to determine whether the endogenous mir-15a, mir-21, or mir-23b miRNAs, respectively, could repress luciferase reporter gene expression.

The pGL3-mir-15a sensor plasmid was created by cloning the sequence (CACAAACCATTATGTGCTGCTA; SEQ ID NO: 369), complementary to the mir-15a miRNA, into the Xba site of the pGL3-Control plasmid, placing the potential miRNA-binding site in the 3'UTR of the luciferase reporter. This reporter plasmid was used to transfect HeLa cells and it was observed that the endogenous mir-15a miRNA was able to inhibit luciferase expression from the pGL3-mir-15a sensor plasmid. Thus, to further evaluate the ability of the mir-15a miRNA to bind this target site encoded by the pGL3-mir-15a sensor plasmid, and to assess the ability of oligomeric compounds to interfere with mir-15a-mediated silencing, pGL3-mir-15a sensor-expressing HeLa cells were treated with varying concentrations (3-, 10- or 30 nM) of the following oligomeric compounds: ISIS Number 327951 (SEQ ID NO: 369) is a uniform 2'-MOE compound targeting the mature mir-15a-1 miRNA. ISIS Numbers 356213 (SEQ ID NO: 878), 356215 (SEQ ID NO: 879), 356216 (SEQ ID NO: 880), 356218 (SEQ ID NO: 881), 356221 (SEQ ID NO: 882), 356227 (SEQ ID NO: 883) and 356229 (SEQ ID NO: 884) are phosphorothioate, uniform 2'-MOE oligomeric compounds designed and synthesized to target the entire length of the mir-15a pri-miRNA molecule (described in detail in Example 28, below). The uniform 2'-MOE phosphorothioate oligomeric compounds ISIS Number 327901 (SEQ ID NO: 319), targeting an unrelated miRNA (mir-143) and ISIS Number 342673 (AGACTAGCGGTATCTTTATCCC; herein incorporated as SEQ ID NO: 758), containing 15 mismatches with respect to the mature mir-143 miRNA, were used as negative controls. The data presented in Table 42 are the average of three trials and are presented as percent untreated control (luciferase plasmid only, not treated with oligomeric compound) luciferase expression, normalized to pRL-CMV levels.

Table 42

Effects of oligomeric compounds on mir-15a miRNA-mediated inhibition of luciferase expression

Treatment	SEQ ID NO	Relative luciferase activity		
		Dose of oligomeric compound		
		3 nM	10 nM	30 nM
327901 negative control	319	83.6	96.6	88.2
342673 negative control	758	104.5	82.6	85.7
327951	369	151.0	207.6	137.1
356213	878	101.2	80.5	109.9
356215	879	98.0	116.7	79.6
356216	880	102.8	84.7	113.2
356218	881	91.6	110.3	85.7
356221	882	106.8	74.0	81.2
356227	883	86.1	117.8	101.5
356229	884	109.7	100.3	97.5

From these data, it was observed that the oligomeric compound ISIS Number 327951 targeting the mature mir-15a miRNA blocked the inhibitory effect of mir-15a, exhibited as a recovery and increase in luciferase activity in HeLa cells expressing the pGL3-mir-15a sensor plasmid.

The pGL3-mir-23b sensor plasmid was created by cloning the sequence (GTGGTAATCCCTGGCAATGTGAT; SEQ ID NO: 307), representing a sequence complementary to the mir-23b miRNA, into the Xba site of the pGL3-Control plasmid, placing the potential miRNA-binding site in the 3'UTR of the luciferase reporter. This reporter plasmid was used to transfect HeLa cells and it was observed that the endogenous mir-23b miRNA was able to inhibit luciferase expression from the pGL3-mir-23b sensor plasmid. Thus, to further evaluate the ability of the mir-23b miRNA to bind this target site encoded by the pGL3-mir-23b sensor plasmid, and to assess the ability of oligomeric compounds to interfere with mir-23b-mediated silencing, pGL3-mir-23b sensor-expressing HeLa cells were treated with varying concentrations (1.3-, 5- or 20 nM) of the following oligomeric compounds: ISIS Number 327889 (SEQ ID NO: 307), a phosphorothioate uniform 2'-MOE oligomeric compound, and ISIS Number 340925 (SEQ ID NO: 307), a 2'-MOE 5-10-8 gapmer oligomeric compound, both targeting mir-23b. The uniform 2'-MOE phosphorothioate oligomeric compound ISIS Number 327924 (SEQ ID NO: 342) targeting an unrelated miRNA (mir-129-2) was used as a negative control. The data are the average of three trials, and are presented in Table 43 as relative luciferase activity (normalized to pRL-CMV luciferase plasmid only, not treated with oligomeric compound).

Table 43

Effects of oligomeric compounds on mir-23b miRNA-mediated inhibition of luciferase expression

Treatment	SEQ ID NO	Fold change luciferase		
		Dose of oligomeric compound		
		1.3 nM	5 nM	20 nM
327924 negative control	342	1.15	0.68	0.92
327889-uniform MOE	307	3.75	3.46	7.40
340925-gapmer	307	0.99	1.41	1.19

From these data, it was observed that, at all doses, ISIS Number 327889, the uniform 2'-MOE oligomeric compound targeting the mature mir-23b miRNA, de-repressed the expression of the luciferase reporter. Thus, ISIS 327889 reversed the silencing effect of the mir-23b miRNA, apparently by inhibiting the binding of mir-23b to its target site encoded by the pGL3-mir-23b sensor plasmid.

The pGL3-mir-21 sensor plasmid was created by cloning the sequence (TCAACATCAGTCTGATAAGCTA; SEQ ID NO: 335), representing a sequence complementary to the mir-21 miRNA, into the Xba site of the pGL3-Control plasmid, placing the potential miRNA-binding site in the 3'UTR of the luciferase reporter. This reporter plasmid was used to transfect HeLa cells and it was observed that the endogenous mir-21 miRNA was able to inhibit luciferase expression from the pGL3-mir-21 sensor plasmid. Thus, to further evaluate the ability of the mir-21 miRNA to bind this target site encoded by the pGL3-mir-21 sensor plasmid, and to assess the ability of oligomeric compounds to interfere with mir-21-mediated silencing, pGL3-mir-21 sensor-expressing HeLa cells were treated with varying concentrations (10 nM or 50 nM) of the following oligomeric compounds: ISIS Number 327917 (SEQ ID NO: 335), a phosphorothioate uniform 2'-MOE oligomeric compound; ISIS Number 338697 (TGCCATGAGATTCAACAGTC; herein incorporated as SEQ ID NO: 524), a uniform 2'-MOE oligomeric compound targeting the mir-21 pri-miRNA molecule; and ISIS Number 328415 (SEQ ID NO: 524), a 2'-MOE 5-10-5 gapmer oligomeric compound targeting the mir-21 pri-miRNA. The uniform 2'-MOE phosphorothioate oligomeric compound ISIS Number 327901 (SEQ ID NO: 319) targeting an unrelated miRNA (mir-143) was used as a negative control. The data are the average of three trials and are presented in Table 44 as percent untreated control (luciferase plasmid only, not treated with oligomeric compound) luciferase expression, normalized to pRL-CMV levels.

Table 44

Effects of oligomeric compounds on

mir-21 miRNA-mediated inhibition of luciferase expression

Treatment	SEQ ID NO	% UTC	
		Dose of oligomeric compound	
		10 nM	50 nM
327901 negative control	319	74.2	83.1
327917	335	1037.6	847.5
338697	524	87.0	84.8
328415	524	66.0	104.4

From these data, it was observed that, at both doses, treatment of HeLa cells with ISIS Number 327917, the uniform 2'-MOE oligomeric compound targeting the mature mir-21 miRNA, de-repressed the expression of the luciferase reporter. Thus, ISIS 327917 reversed the silencing effect of the endogenous mir-21 miRNA, apparently by inhibiting the binding of mir-21 to its target site encoded by the pGL3-mir-21 sensor plasmid.

Therefore, oligomeric compounds targeting and/or mimicking the mir-143, mir-15a, mir-23b and mir-21 miRNAs and their corresponding pri-miRNA molecules have been demonstrated to bind to target RNA transcripts and silence reporter gene expression.

Example 28: Effects of oligomeric compounds on expression of pri-miRNAs

As described above in Example 19, pri-miRNAs, often hundreds of nucleotides in length, are processed by a nuclear enzyme in the RNase III family known as Drosha, into approximately 70 nucleotide-long pre-miRNAs (also known as stem-loop structures, hairpins, pre-mirs or foldback miRNA precursors), and pre-miRNAs are subsequently exported from the nucleus to the cytoplasm, where they are processed by human Dicer into double-stranded miRNAs, which are subsequently processed by the Dicer RNase into mature miRNAs. It is believed that, in processing the pri-miRNA into the pre-miRNA, the Drosha enzyme cuts the pri-miRNA at the base of the mature miRNA, leaving a 2-nt 3'overhang (Lee, et al., Nature, 2003, 425, 415-419). The 3' two-nucleotide overhang structure, a signature of RNaseIII cleavage, has been identified as a critical specificity determinant in targeting and maintaining small RNAs in the RNA interference pathway (Murchison, et al., Curr. Opin. Cell Biol., 2004, 16, 223-9).

The oligomeric compounds of the present invention are believed to disrupt pri-miRNA and/or pre-miRNA structures, and sterically hinder Drosha and/or Dicer cleavage, respectively. Additionally, oligomeric compounds capable of binding to the mature miRNA are believed to prevent the RISC-mediated binding of a miRNA to its mRNA target, either by cleavage or steric occlusion of the miRNA.

Using the real-time RT-PCR methods described in Example 19, the expression levels of the mir-15a pri-miRNA were compared in HepG2 cells treated with a nested series of chimeric gapmer oligomeric compounds, targeting and spanning the entire length of the mir-15a pri-miRNA; these compounds are shown in Table 45, below. Each gapmer is 20 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide "wings." The wings are composed of 2'-methoxyethoxy (2'-MOE) nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines. Using the transfection methods described herein, HepG2 cells were treated with 100 nM of each of these gapmer oligomeric compounds. Total RNA was isolated from HepG2 cells by lysing cells in 1 mL TRIZOL™ (Invitrogen) using the manufacturer's recommended protocols. Real-time RT-PCR analysis was performed using a primer/probe set specific for the mir-15a pri-miRNA molecule to assess the effects of these compounds on expression of the mir-15a pri-miRNA molecule. ISIS 339317 (GTGTGTTTAAAAAAAATAAAACCTTGGA; SEQ ID NO.: 885) was used as the forward primer, ISIS 339318 (TGGCCTGCACCTTTTCAAA; SEQ ID NO.: 886) was used as the reverse primer, and ISIS 339319 (AAAGTAGCAGCAC ATAATGGTTTGTGG; SEQ ID NO.: 887) was used as the probe. Total RNA was quantified using RiboGreen™ RNA quantification reagent (Molecular Probes, Inc. Eugene, OR), expression levels observed for each target are normalized to 5.8S rRNA, and values are expressed relative to the untreated control. Inhibition of expression of the mir15a pri-miRNA by these gapmer oligomeric compounds is expressed as a percentage of RNA levels in untreated control cells. Results of these experiments are described in Table 45 below:

Table 45

Effects of chimeric oligomeric compounds on expression of the mir-15a pri-miRNA

ISIS Number	SEQ ID NO	Sequence	Expression of mir-15a pri-miRNA (%UTC)
347964	878	TATAACATTGATGTAATATG	13.7
347965	888	GCTACTTTACTCCAAGGTTT	86.0
347966	879	TGCTACTTTACTCCAAGGTT	39.2
347967	880	GCACCTTTTCAAAATCCACA	152.3
347968	889	CCTGCACCTTTTCAAAATCC	8.4
347969	881	TGGCCTGCACCTTTTCAAAA	39.5
347970	890	ATATGGCCTGCACCTTTTCA	2.2
347971	891	ACAATATGGCCTGCACCTTT	92.8
347972	882	AGCACAATATGGCCTGCACC	98.6
347973	892	GGCAGCACAATATGGCCTGC	143.3
347974	893	TGAGGCAGCACAATATGGCC	98.1
347975	894	TTTTGAGGCAGCACAATATG	9.2
347976	895	TATTTTTGAGGCAGCACAAT	73.0
347977	896	TTGTATTTTTGAGGCAGCAC	111.3

347978	883	TCCTTGTATTTTTGAGGCAG	51.1
347979	897	AGATCCTTGTATTTTTGAGG	74.9
347980	884	AGATCAGATCCTTGTATTTT	3.6
347981	898	AGAAGATCAGATCCTTGTAT	N/D
347982	899	TTCAGAAGATCAGATCCTTG	82.2
347983	900	AAATATATTTTCTTCAGAAG	13.0

From these data, it was observed that oligomeric compounds ISIS Numbers 347964, 347966, 347968, 347970, 347975, 347980 and 347983 show significant inhibition of expression of the mir-15a pri-miRNA molecule. Thus, it is believed that the antisense oligomeric compounds ISIS Numbers 347964, 347966, 347968, 347970, 347975, 347980 and 347983 bind to the mir-15a pri-miRNA and/or pre-miRNA molecules and cause their degradation and cleavage.

From these data, it was observed that oligomeric compounds ISIS Numbers 347967, 347977 and 347973 stimulate an increase in expression levels of the mir-15a pri-miRNA. It is believed that the oligomeric compounds ISIS Numbers 347967, 347977 and 347973 bind to the mir-15a pri-miRNA and inhibit its processing into the mature mir-15a miRNA. It is believed that, in addition to the increase in the levels of the mir-15a pri-miRNA observed upon treatment of cells with the oligomeric compounds ISIS Numbers 347977, 347967 and 347973, a drop in expression levels of the fully processed mature mir-15a miRNA may also trigger a feedback mechanism signaling these cells to increase production of the mir-15a pri-miRNA.

The gapmer oligomeric compounds targeting the mir-15b and mir-15-a-1 mature miRNAs described above were also transfected into T47D cells according to standard procedures. In addition, uniform 2'-MOE and 2'-MOE gapmer oligomeric compounds targeting the mature mir-15a-1 and mir-15b miRNAs were also transfected into T47D cells, for analysis of their effects on mir-15a-1 and mir-15b pri-miRNA levels. The oligomeric compounds ISIS Number 327927 (SEQ ID NO: 345), a uniform 2'-MOE compound and ISIS Number 345391 (SEQ ID NO: 345), a 2'-MOE 5-10-7 gapmer compound, both target mir-15b. The oligomeric compounds ISIS Number 327951 (SEQ ID NO: 369), a uniform 2'-MOE compound, and ISIS Number 345411 (SEQ ID NO: 369), a 2'-MOE 5-10-7 gapmer compound, both target mir-15a-1. Oligomeric compounds ISIS Number 129686 (CGTTATTAACCTCCGTTGAA; SEQ ID NO: 901), and ISIS Number 129691 (ATGCATACTACGAAAGGCCG; SEQ ID NO:902), both universal scrambled controls, as well as ISIS Number 116847 (CTGCTAGCCTCTGGATTTGA; SEQ ID NO: 844) targeting an unrelated gene, PTEN, were used as negative controls. ISIS Numbers 129686, 129691, and 116847 are phosphorothiated 2'-MOE 5-10-5 gapmers, and all cytosines are 5-methylcytosines. T47D cells (seeded in 12-well plates) were treated with these

oligomeric compounds, and RNA was isolated from the treated cells by lysing in 1 mL TRIZOL™ (Invitrogen) and total RNA was prepared using the manufacturer's recommended protocols. To assess the effects of these compounds on expression of the mir-15a or mir-15b pri-miRNA molecules, real-time RT-PCR analysis was performed using either the primer/probe set specific for the mir-15a pri-miRNA molecule described above, or a primer probe set specific for the mir-15b pri-miRNA molecule: ISIS 339320 (CCTACATTTTTGAGGCCTTAAAGTACTG; SEQ ID NO: 903) was used as the forward primer for the mir-15b pri-miRNA, ISIS 339321 (CAAATAATGATTCGCATCTTGACTGT; SEQ ID NO: 904) was used as the reverse primer for the mir-15b pri-miRNA, and ISIS 339322 (AGCAGCACATCATGGTTTACATGC; SEQ ID NO: 905) was used as the probe. Total RNA was quantified using RiboGreen™ RNA quantification reagent (Molecular Probes, Inc. Eugene, OR), expression levels observed for each target were normalized to 5.8S rRNA, and values are expressed relative to the untreated control. Inhibition of expression of the mir15a or mir-15b pri-miRNA molecules upon treatment with these oligomeric compounds is was assessed and expressed as a percentage of RNA levels in untreated control cells.

On multiple repeats of these experiments, it was observed that the uniform 2'-MOE oligomeric compounds ISIS Number 327927 (SEQ ID NO: 345) and ISIS Number 327951 (SEQ ID NO: 369), targeted to the mature mir-15b and mir-15a-1 miRNAs, respectively, each stimulate an approximately 2.5- to 3.5-fold increase in expression of the mir-15a pri-miRNA molecule and an approximately 1.5- to 2.5-fold increase in the expression of the mir-15b pri-miRNA molecule. Therefore, it is believed that ISIS Numbers 327927 and 327951 can bind to the mir-15a and/or mir-15b pri-miRNA or pre-miRNA molecules and interfere with their processing into the mature mir-15a or mir-15b miRNAs. It is also recognized that a decrease in levels of the mature, processed forms of the mir-15a or mir-15b miRNAs in T47D cells treated with ISIS Number 345411 (SEQ ID NO: 369), ISIS Number 327927 (SEQ ID NO: 345) or ISIS Number 327951 (SEQ ID NO: 369) may also trigger a feedback mechanism that signals these cells to increase production of the mir-15a and/or mir-15b pri-miRNA molecules.

In accordance with the present invention, a nested series of uniform 2'-MOE oligomeric compounds were designed and synthesized to target the entire length of the mir-15a pri-miRNA molecule. Each compound is 19 nucleotides in length, composed of 2'-methoxyethoxy (2'-MOE) nucleotides and phosphorothioate (P=S) internucleoside linkages throughout the oligonucleotide. All cytidine residues are 5-methylcytidines. The compounds are shown in Table 46. The compounds can be analyzed for their effect on mature miRNA, pre-miRNA or pri-miRNA levels

by quantitative real-time PCR, or they can be used in other assays to investigate the role of miRNAs or the function of targets downstream of miRNAs.

Table 46

Uniform 2'-MOE PS Compounds targeting the mir-15a pri-miRNA

ISIS Number	SEQ ID NO	Sequence
356213	878	TATAACATTGATGTAATATG
356214	879	GCTACTTTACTCCAAGGTTT
356215	880	TGCTACTTTACTCCAAGGTT
356216	881	GCACCTTTTCAAAATCCACA
356217	882	CCTGCACCTTTTCAAAATCC
356218	883	TGGCCTGCACCTTTTCAAAA
356219	884	ATATGGCCTGCACCTTTTCA
356220	888	ACAATATGGCCTGCACCTTT
356221	889	AGCACAATATGGCCTGCACC
356222	890	GGCAGCACAATATGGCCTGC
356223	891	TGAGGCAGCACAATATGGCC
356224	892	TTTTGAGGCAGCACAATATG
356225	893	TATTTTTGAGGCAGCACAAT
356226	894	TTGTATTTTTGAGGCAGCAC
356227	895	TCCTTGTATTTTTGAGGCAG
356228	896	AGATCCTTGTATTTTTGAGG
356229	897	AGATCAGATCCTTGTATTTT
356230	898	AGAAGATCAGATCCTTGTAT
356231	899	TTCAGAAGATCAGATCCTTG
356232	900	AAATATATTTTCTTCAGAAG

Using the real-time RT-PCR methods described, the expression levels of the mir-15a pri-miRNA were compared in T47D cells treated with the nested series of uniform 2'-MOE oligomeric compounds, targeting and spanning the entire length of the mir-15a pri-miRNA. The region encompassing the mir-15a primary transcript (the complement of nucleotides 31603159 to 31603468 of GenBank Accession number NT_024524.13; AAATAATTATGCATATTACAT CAATGTTATAATGTTTAAACATAGATTTTTTTACATGCATTCTTTTTTTCCTGAAAGA AAATATTTTTTATATTCTTTAGGCGCGAATGTGTGTTTAAAAAAAATAAAACCTTGG AGTAAAGTAGCAGCACATAATGGTTTGTGGATTTTGAAAAGGTGCAGGCCATATTGT GCTGCCTCAAAAATACAAGGATCTGATCTTCTGAAGAAAATATATTTCTTTTTATTC ATAGCTCTTATGATAGCAATGTCAGCAGTGCCTTAGCAGCACGTAAATATTGGCGTT AAG) is incorporated herein as SEQ ID NO: 906. ISIS Number 356215 (SEQ ID NO: 879) targets a region flanking and immediately 5′ to the predicted 5′ Drosha cleavage site in the mir-15a pri-miRNA. ISIS Number 356218 (SEQ ID NO: 881) targets a region in the loop of the mir-15a pri-miRNA. ISIS 356227 (SEQ ID NO: 883) targets a region flanking and immediately 3′ to the predicted 3′ Drosha cleavage site in the mir-15a pri-miRNA. Additionally, oligomeric compound ISIS 327951 (SEQ ID NO: 369), a uniform 2'-MOE compound targeting the mature

mir-15a-1 miRNA, was tested for comparison. Oligomeric compounds ISIS 327901 (SEQ ID NO: 319) targeting the mature mir-143 miRNA; ISIS 129690, (TTAGAATACGTCGCG TTATG; SEQ ID NO: 907), a phosphorothioate 5-10-5 MOE gapmer used as a universal scrambled control; and ISIS 116847 (CTGCTAGCCTCTGGATTTGA; SEQ ID NO: 844), a uniform 5-10-5 2'-MOE gapmer targeting an unrelated gene, PTEN, were used as negative controls. Using the transfection methods previously described, T47D cells were treated with 100 nM of each of these oligomeric compounds. Total RNA was isolated by lysing cells in 1 mL TRIZOL™ (Invitrogen) using the manufacturer's recommended protocols. real-time RT-PCR analysis was performed using a primer/probe set specific for the mir-15a pri-miRNA molecule [forward primer=ISIS 339317 (SEQ ID NO.: 885), reverse primer=ISIS 339318 (SEQ ID NO.: 886), and probe=ISIS 339319 (SEQ ID NO.: 887)]. Total RNA was quantified using RiboGreen™ RNA quantification reagent (Molecular Probes, Inc. Eugene, OR), expression levels observed for each target were normalized to 5.8S rRNA, and values were expressed relative to the untreated control (UTC). Effects on expression of the mir-15a pri-miRNA molecule resulting from treatment of T47D cells with these uniform 2'-MOE oligomeric compounds is expressed as a percentage of RNA levels in untreated control cells. Results of these experiments are described in Table 47 below:

Table 47

Effects of uniform 2'-MOE oligomeric compounds on mir-15a pri-miRNA expression

ISIS #	SEQ ID NO:	target	% UTC
UTC	N/A	N/A	100
129690 scrambled control	XXX	N/A	121
327901	319	mir-143	132
116847	844	PTEN mRNA	132
327951	369	mature mir-15a-1	713
356213	878	>100 bp upstream of mature mir-15a	171
356215	879	flanking 5' Drosha cleavage site of mir-15a-1 pri-miRNA	1005
356216	880	mir-15a-1 pri-miRNA	503
356218	881	loop of mir-15a-1 pri-miRNA	392
356221	882	mir-15a-1 pri-miRNA	444
356224	894	mir-15a-1 pri-miRNA	592
356227	883	flanking 3' Drosha cleavage site of mir-15a-1 pri-miRNA	879
356229	884	mir-15a-1 pri-miRNA	818
356231	899	mir-15a-1 pri-miRNA	811
356232	900	mir-15a-1 pri-miRNA	631

From these data, it was observed that the uniform 2'-MOE oligomeric compounds ISIS Numbers 327927, 327951, 356215, 356216, 356218, 356221, 356224, 356227, 356229, 356231 and 356232 stimulate an increase in levels of the mir-15a pri-miRNA molecule as detected by

real-time RT-PCR. Notably, oligomeric compounds ISIS Numbers 356215 and 356227 which target the regions immediately flanking the predicted 5′ and 3′ Drosha cleavage sites in the mir-15a pri-miRNA, respectively, were observed to stimulate the greatest increases in expression of the mir-15a pri-miRNA. It is believed that these oligomeric compounds bind to the mir-15a pri-miRNA and/or pre-miRNA molecules and interfere with their processing into the mature mir-15a miRNA, possibly by interfering with the activity of RNase III-like enzymes such as human Dicer and/or Drosha. The resultant decrease in levels of the processed mature mir-15a miRNA may trigger a feedback mechanism leading to an upregulation of production of the mir-15a pri-miRNA molecule. Not mutually exclusive with the processing interference and the feedback mechanisms is the possibility that treatment with oligomeric compounds could stimulate the activity of an RNA-dependent RNA polymerase (RdRP) that amplifies the mir-15a pri-miRNA or pre-miRNA molecules. It is understood that such oligomeric compound-triggered mechanisms may be operating not only upon regulation of mir-15a production and processing, but may also be found to regulate the production and processing of other miRNAs.

The expression levels of mir-24-2, let-7i, and let-7d were assessed in HeLa or T-24 cells treated with various uniform 2'-MOE oligomeric compounds targeting mature miRNAs. For example, using the transfection methods previously described, HeLa cells were treated with 100 nM of the oligomeric compound ISIS Number 327945 (SEQ ID NO: 363) targeting the mir-24-2 mature miRNA. Total RNA was isolated and expression levels of the mir-24-2 pri-miRNA were analyzed by real-time quantitative RT-PCR using a primer/probe set specific for the mir-24-2 pri-miRNA molecule (forward primer=ISIS 359358 (CCCTGGGCTCTGCCT; herein incorporated as SEQ ID NO.: 908), reverse primer=ISIS 359359 (TGTACACAAACCAAC TGTGTTTC; herein incorporated as SEQ ID NO.: 909), and probe=ISIS 359360 (CGTGCC TACTGAGC; herein incorporated as SEQ ID NO.: 910)). An approximately 35-fold increase in expression levels of the mir-24-2 pri-miRNA molecule was observed in HeLa cells treated with the oligomeric compound ISIS 327945 as detected by real-time RT-PCR.

Using the transfection methods previously described, HeLa cells were treated with 100 nM of the oligomeric compound ISIS Number 327890 (SEQ ID NO: 308) targeting the let-7i mature miRNA. Total RNA was isolated and expression levels of the let-7i pri-miRNA were analyzed by real-time quantitative RT-PCR using a primer/probe set specific for the let-7i pri-miRNA molecule (forward primer=ISIS 341684 (TGAGGTAGTAGTTTGTGCTGTTGGT; herein incorporated as SEQ ID NO.: 777), reverse primer=ISIS 341685 (AGGCAGTAGCTTGCGCAGTTA; herein incorporated as SEQ ID NO.: 778), and probe=ISIS 341686 (TTGTGACATTGCCCGCTGTGGAG; herein incorporated as SEQ ID NO.: 779)). An

approximately 4-fold increase in expression levels of the let-7i pri-miRNA molecule was observed in HeLa cells treated with the oligomeric compound ISIS 327890 as detected by real-time RT-PCR.

Using the transfection methods previously described, *supra*, T-24 cells were treated with 100 nM of the oligomeric compound ISIS Number 327926 (SEQ ID NO: 344) targeting the let-7d mature miRNA. Total RNA was isolated and expression levels of the let-7d pri-miRNA were analyzed by real-time quantitative RT-PCR using a primer/probe set specific for the let-7d pri-miRNA molecule (forward primer=ISIS 341678 (CCTAGGAAGAGGTAG TAGGTTGCA; herein incorporated as SEQ ID NO.: 771), reverse primer=ISIS 341679 (CAGCAGGTCGTATAGTTACCTCCTT; herein incorporated as SEQ ID NO.: 772), and probe=ISIS 341680 (AGTTTTAGGGCAGGGATTTTGCCCA; herein incorporated as SEQ ID NO.: 773)). An approximately 1.7-fold increase in expression levels of the let-7d pri-miRNA molecule was observed in T-24 cells treated with the oligomeric compound ISIS 327926 as detected by real-time RT-PCR.

Thus, treatment with uniform 2'-MOE oligomeric compounds targeting mature miRNAs appears to result in an induction of expression of the corresponding pri-miRNA molecule.

In one embodiment, the expression of mir-21 (noted to be expressed at high levels in HeLa cells) was assessed in cells treated with oligomeric compounds. Using the transfection methods previously described, HeLa cells were treated with 100 nM of the uniform 2'-MOE oligomeric compound ISIS Number 327917 (SEQ ID NO: 335) targeting the mir-21 mature miRNA. Total RNA was isolated by lysing cells in 1 mL TRIZOL™ (Invitrogen) using the manufacturer's recommended protocols. By Northern blot analysis of total RNA from HeLa cells treated with ISIS 327917, expression levels of the mir-21 mature miRNA were observed to be reduced to 50% of those of untreated control cells. Furthermore, expression levels of the mir-21 pri-miRNA were found to increase in these HeLa cells treated with the oligomeric compound ISIS 327917. Real-time RT-PCR analysis was also performed on HeLa cells treated with ISIS 327917 using a primer/probe set specific for the mir-21 pri-miRNA molecule [forward primer=ISIS 339332 (GCTGTACCACCTTGTCGGGT; herein incorporated as SEQ ID NO.: 911), reverse primer=ISIS 339333 (TCGACTGGTGTTGCCATGA; herein incorporated as SEQ ID NO.: 912), and probe=ISIS 339334 (CTTATCAGACTGATGTTGACTGTTGAAT; herein incorporated as SEQ ID NO.: 913)]. Total RNA was quantified using RiboGreen™ RNA quantification reagent (Molecular Probes, Inc. Eugene, OR), expression levels observed for the target were normalized to 5.8S rRNA, and values were expressed relative to an untreated control (UTC). ISIS Number 327917 was observed to stimulate an approximately 2-fold increase in

levels of the mir-21 pri-miRNA molecule as detected by real-time RT-PCR.

Thus, it is believed that, in addition to binding the mir-21 mature miRNA and interfering with the RISC-mediated binding of mir-21 to its mRNA target, the oligomeric compound, ISIS 327917, binds to the mir-21 pri-miRNA and/or pre-miRNA molecules and interferes with their processing into the mature mir-21 miRNA, inhibiting expression of the mature mir-21 miRNA in HeLa cells, possibly by interfering with the activity of RNase III-like enzymes such as human Dicer or Drosha. The resultant decrease in levels of mature mir-21 miRNA may trigger a feedback mechanism leading to an upregulation of production of the mir-21 pri-miRNA molecule. Treatment with this oligomeric compound could also stimulate the activity of an RNA-dependent RNA polymerase (RdRP) that amplifies the mir-21 pri-miRNA or pre-miRNA molecules.

In accordance with the present invention, a nested series of uniform 2'-MOE oligomeric compounds were designed and synthesized to target the entire length of the mir-21 pri-miRNA molecule. The region encompassing the mir-21 primary transcript (nucleotides 16571584 to 16571864 of GenBank Accession number NT_010783.14; CTGGGTTTTTTTGGTTTGT TTTTGTTTTTGTTTTTTTTATCAAATCCTGCCTGACTGTCTGCTTGTTTTGCCTACCATC GTGACATCTCCATGGCTGTACCACCTTGTCGGGTAGCTTATCAGACTGATGTTGACT GTTGAATCTCATGGCAACACCAGTCGATGGGCTGTCTGACATTTTGGTATCTTTCATC TGACCATCCATATCCAATGTTCTCATTTAAACATTACCCAGCATCATTGTTTATAATC AGAAACTCTGGTCCTTCTGTCTGGTGGCAC) is incorporated herein as SEQ ID NO: 914. Each compound is 20 nucleotides in length, composed of 2'-methoxyethoxy (2'-MOE) nucleotides and phosphorothioate (P=S) internucleoside linkages throughout the compound. All cytidine residues are 5-methylcytidines. The compounds are shown in Table 48. The compounds can be analyzed for their effect on mature miRNA, pre-miRNA or pri-miRNA levels by quantitative real-time PCR, or they can be used in other assays to investigate the role of miRNAs or the function of targets downstream of miRNAs.

Table 48

Uniform 2'-MOE PS Compounds targeting the mir-21 pri-miRNA

ISIS Number	SEQ ID NO	Sequence
358765	915	ACAAGCAGACAGTCAGGCAG
358766	916	GGTAGGCAAAACAAGCAGAC
358767	917	GGAGATGTCACGATGGTAGG
358768	918	AGGTGGTACAGCCATGGAGA
358769	919	GATAAGCTACCCGACAAGGT
358770	920	AGTCTGATAAGCTACCCGAC
358771	921	CAACAGTCAACATCAGTCTG
358772	922	GAGATTCAACAGTCAACATC

358773	923	CTGGTGTTGCCATGAGATTC
358774	924	CATCGACTGGTGTTGCCATG
358775	925	ACAGCCCATCGACTGGTGTT
358776	926	TGTCAGACAGCCCATCGACT
358777	927	CCAAAATGTCAGACAGCCCA
358778	928	GATACCAAAATGTCAGACAG
358779	929	GGTCAGATGAAAGATACCAA
358780	930	AACATTGGATATGGATGGTC
358781	931	TAATGTTTAAATGAGAACAT
358782	932	AACAATGATGCTGGGTAATG
358783	933	GAGTTTCTGATTATAAACAA
358784	934	CGACAAGGTGGTACAGCCAT
358785	935	GAAAGATACCAAAATGTCAG

Using the real-time RT-PCR methods, the expression levels of the mir-21 pri-miRNA were compared in HeLa cells treated with this nested series of uniform 2'-MOE oligomeric compounds, targeting and spanning the entire length of the mir-21 pri-miRNA. ISIS Number 358768 (SEQ ID NO: 918) targets a region flanking the predicted 5′ Drosha cleavage site in the mir-21 pri-miRNA. ISIS Number 358777 (SEQ ID NO: 927) targets a region spanning the 3′ Drosha cleavage site in the mir-21 pri-miRNA. ISIS 358779 (SEQ ID NO: 929) targets a region flanking the predicted 3′ Drosha cleavage site in the mir-21 pri-miRNA. Additionally, oligomeric compounds ISIS 327917 (SEQ ID NO: 335), a uniform 2'-MOE compound targeting the mature mir-21 miRNA, and ISIS 345382 (TCAACATCAGTCTGATAAGCTA; SEQ ID NO: 335), a 5-10-7 phosphorothioate 2'-MOE gapmer targeting mir-21, were tested for comparison. Oligomeric compound ISIS 327863 (ACGCTAGCCTAATAGCGAGG; herein incorporated as SEQ ID NO: 936), a phosphorothioate 5-10-5 2'-MOE gapmer, was used as scrambled control. Using the transfection methods previously described, HeLa cells were treated with 100 nM of each of these oligomeric compounds. Total RNA was isolated by lysing cells in 1 mL TRIZOL™ (Invitrogen) using the manufacturer's recommended protocols. real-time RT-PCR analysis was performed using the primer/probe set specific for the mir-21 pri-miRNA molecule [forward primer=ISIS 339332 (SEQ ID NO.: 911), reverse primer=ISIS 339333 (SEQ ID NO.: 912), and probe=ISIS 339334 (SEQ ID NO.: 913)]. Total RNA was quantified using RiboGreen™ RNA quantification reagent (Molecular Probes, Inc. Eugene, OR), expression levels observed for each target were normalized to 5.8S rRNA, and values were expressed relative to the untreated control (UTC). Effects on expression of the mir-21 pri-miRNA molecule resulting from treatment of HeLa cells with these uniform 2'-MOE oligomeric compounds is expressed as a percentage of RNA levels in untreated control cells. Results of these experiments are shown in Table 49 below:

Table 49

Effects of oligomeric compounds on mir-21 pri-miRNA expression

ISIS #	SEQ ID NO:	target	% UTC
UTC	N/A	N/A	100
327863 gapmer control	936	N/A	107
327917 uniform 2'-MOE	335	mature mir-21	249
345382 5-10-7 2'-MOE gapmer	335	mature mir-21	119
358765	915	mir-21 pri-miRNA	133
358766	916	mir-21 pri-miRNA	142
358767	917	mir-21 pri-miRNA	248
358768	918	flanking 5' Drosha cleavage site of mir-21 pri-miRNA	987
358769	919	mir-21 pri-miRNA	265
358770	920	mir-21 pri-miRNA	250
358771	921	mir-21 pri-miRNA	181
358772	922	mir-21 pri-miRNA	245
358773	923	mir-21 pri-miRNA	148
358774	924	mir-21 pri-miRNA	104
358775	925	mir-21 pri-miRNA	222
358776	926	mir-21 pri-miRNA	367
358777	927	spanning 3' Drosha cleavage site of mir-21 pri-miRNA	536
358778	928	mir-21 pri-miRNA	503
358779	929	flanking 3' Drosha cleavage site of mir-21 pri-miRNA	646
358780	930	mir-21 pri-miRNA	269
358781	931	mir-21 pri-miRNA	122
358782	932	mir-21 pri-miRNA	155
358783	933	mir-21 pri-miRNA	133
358784	934	mir-21 pri-miRNA	358
358785	935	mir-21 pri-miRNA	257

From these data, it was observed that the uniform 2'-MOE oligomeric compounds ISIS Numbers 327917, 358767, 358768, 358769, 358770, 358772, 358775, 358776, 358777, 358778, 358779, 358780, 358784 and 358785 stimulate an increase in levels of the mir-21 pri-miRNA molecule as detected by real-time RT-PCR. Notably, oligomeric compounds ISIS Numbers 358768 and 358779 which target the regions flanking the predicted 5' and 3' Drosha cleavage sites, respectively, and ISIS Number 358777, which targets a region spanning the 3' Drosha cleavage site in the mir-21 pri-miRNA were observed to stimulate the greatest increases in expression of the mir-21 pri-miRNA. Furthermore, treatment of HeLa cells with increasing concentrations (25, 50, 100, and 200 nM) of ISIS Numbers 358768, 358779, and 327917 was observed to result in a dose-responsive induction of mir-21 pri-miRNA levels. Thus, it is believed that these oligomeric compounds bind to the mir-21 pri-miRNA and/or pre-miRNA molecules and interfere with their processing into the mature mir-21 miRNA, possibly by interfering with the activity of RNase III-like enzymes such as human Dicer and/or Drosha. The resultant

decrease in levels of the processed mature mir-21 miRNA may trigger a feedback mechanism leading to an upregulation of production of the mir-21 pri-miRNA molecule. Not mutually exclusive with the processing interference and the feedback mechanisms is the possibility that treatment with oligomeric compounds could stimulate the activity of an RNA-dependent RNA polymerase (RdRP) that amplifies the mir-21 pri-miRNA or pre-miRNA molecules. It is understood that such oligomeric compound-triggered mechanisms may be operating not only upon regulation of mir-21 production and processing, but may also be found to regulate the production and processing of other miRNAs or target nucleic acids.

In one embodiment, the oligomeric compounds ISIS Number 327917 (SEQ ID NO: 335), the phosphorothioate uniform 2'-MOE targeting mature mir-21; ISIS Number 358768 (SEQ ID NO: 918), the uniform 2'-MOE targeting the mir-21 pri-miRNA which stimulated the largest increase in pri-miRNA expression levels by real time quantitative RT-PCR; and ISIS Number 345382 (SEQ ID NO: 335), the 5-10-7 phosphorothioate 2'-MOE gapmer targeting mature mir-21 were selected for dose response studies in HeLa cells using the luciferase reporter system described in Example 27. ISIS Number 342683 (SEQ ID NO: 790), representing the scrambled nucleotide sequence of an unrelated PTP1B antisense oligonucleotide, was used as a negative control. HeLa cells expressing the pGL3-mir-21 sensor plasmid (described in Example 27) were treated with 1.9, 5.5, 16.7, and 50 nM of these oligomeric compounds, to assess the ability of oligomeric compounds to interfere with endogenous mir-21-mediated silencing of the pGL3-mir-21 sensor plasmid. The data are presented in Table 50 as percent untreated control (luciferase plasmid only, not treated with oligomeric compound) luciferase expression, normalized to pRL-CMV levels.

Table 50

Effects of oligomeric compounds on mir-21 miRNA-mediated inhibition of luciferase expression

Treatment	% UTC			
	Dose of oligomeric compound			
	1.9 nM	5.5 nM	16.7 nM	50 nM
342683 negative control	127	171	104	108
327917	522	1293	2470	4534
358768	103	163	146	118
345382	101	135	117	95

From these data, it was observed that, at all doses, treatment of HeLa cells with ISIS Number 327917, the uniform 2'-MOE oligomeric compound targeting the mature mir-21 miRNA, de-repressed the expression of the luciferase reporter, in a dose-dependent fashion.

Thus, ISIS 327917 reversed the silencing effect of the endogenous mir-21 miRNA, possibly by inhibiting the binding of mir-21 to its target site encoded by the pGL3-mir-21 sensor plasmid.

Example 29: Diseases associated with miRNA-containing loci

Using the public databases Online Mendelian Inheritance in Man (OMIM) (accessible through the Internet at, for example, ftp.ncbi.nih.gov/repository/OMIM/) and LocusLink (accessible at, for example, ftp.ncbi.nlm.nih.gov/refseq/LocusLink/), a bioinformatic analysis was performed which allowed the prediction of miRNAs associated with several human diseases. First, miRNAs encoded within genes having LocusLink identification numbers were identified, and these were compared to tables (for example, "mim2loc," which connects LocusLink identification numbers with OMIM identification numbers, as well as "genemap," "genemap.key," "mim-title," and "morbidmap" tables) for the construction of a new database called "db1.mdb" linking miRNAs to LocusLink and OMIM identification numbers and linking these to human diseases.

It was observed that, beginning with 95 pri-miRNAs, a subset of 49 had OMIM identification numbers, 48 of which were linked to OMIM names. Six of these miRNAs were associated with specific diseased patients (some in each category were duplicates). Thus, the majority of miRNAs with OMIM identification numbers are not directly linked to observed diseases, but are likely to be important in pathways (such as cholesterol homeostasis) associated with diseases. Tables 51 and 52 summarize information retrieved from these studies.

Table 51

miRNA genes associated with specific diseases

OMIM ID:	locus containing miRNA	Disease association:
120150	collagen, type I, alpha 1/ hypothetical miRNA-144	Osteogenesis imperfecta, type I, 166200
114131	calcitonin receptor containing hypothetical miRNA 30	Osteoporosis, postmenopausal susceptibility, 166710
605317	forkhead box P2/ hypothetical miRNA 169	Speech-language disorder-1, 602081
600700	LIM domain-containing preferred translocation partner in lipoma containing miR-28	Lipoma; Leukemia, myeloid
160710	myosin, heavy polypeptide 6, cardiac muscle, alpha (cardiomyopathy, hypertrophic 1) containing miR-208	Cardiomyopathy, familial hypertrophic, 192600
606157	hypothetical protein FLJ11729 containing mir-103-2	Neurodegeneration, pantothenate kinase-associated, 234200

The previous table shows miRNAs associated with an OMIM record that were also associated with diseased patients.

The following table, Table 52, describes diseases or disease-related phenotypes found to be associated with genetic loci associated with a miRNA.

Table 52

miRNAs associated with disease phenotypes

OMIM ID:	Locus containing miRNA	Disease association:
114131	calcitonin receptor containing hypothetical miRNA-30	Osteoporosis, postmenopausal, susceptibility, 166710
120150	collagen, type I, alpha 1/ hypothetical miRNA-144	Osteogenesis imperfecta, type I, 166200
138247	glutamate receptor, ionotropic, AMPA 2 / hypothetical miRNA-171	cerebellar long-term depression
160710	myosin, heavy polypeptide 6, cardiac muscle, alpha (cardiomyopathy, hypertrophic 1) containing miR-208	Cardiomyopathy, familial hypertrophic, 192600
184756	sterol regulatory element-binding protein-1/ mir-33b	Emery-Dreifuss muscular dystrophy, 310300; dilated cardiomyopathy (CMD1A), 115200; familial partial lipodystrophy (FPLD), 151660
300093	gamma-aminobutyric acid (GABA) A receptor, epsilon	early-onset parkinsonism, or Waisman syndrome, 311510; and MRX3 X-linked mental retardation, 309541
305660	gamma-aminobutyric acid (GABA) A receptor, alpha 3 containing miR-105 (Mourelatos) and miR-105-2	manic depressive illness, colorblindness, and G6PD
305915	glutamate receptor, ionotrophic, AMPA 3/ hypothetical miRNA-033	complex bipolar disorder; drug addiction
600150	potassium large conductance calcium-activated channel, subfamily M, alpha member 1 containing hypothetical miRNA-172	cardiovascular disease
600395	glypican 1 containing miR-149	angiogenesis
600481	Sterol regulatory element binding transcription factor 2 containing mir-33a	LDL and cholesterol homeostasis
600592	Minichromosome maintenance deficient (S. cerevisiae) 7 containing miR-93 (Mourelatos) and miR-25 and miR-94	increased chromosomal loss, DNA replication and recombination
600700	LIM domain-containing preferred translocation partner in lipoma containing miR-28	Lipoma; Leukemia, myeloid
600758	Focal adhesion kinase, p125 / mir-151	oncogenesis
601009	tight junction protein 1 (zona occludens 1)/ hypothetical miRNA-183	peptic ulcer disease and gastric carcinoma
601029	mesoderm specific transcript (mouse) homolog containing mir-240* (Kosik)	intrauterine and postnatal growth retardation

601698	protein tyrosine phosphatase, receptor type, N polypeptide 2 containing mir-153-2	insulin-dependent diabetes mellitus (IDDM)
601773	protein tyrosine phosphatase, receptor type, N containing mir-153-1	insulin-dependent diabetes mellitus (IDDM), 222100
603576	melastatin 1 containing mir-211	metastatic human melanoma
603634	ribosomal protein L5/ hypothetical miRNA 168-2	colorectal cancers
603745	slit (Drosophila) homolog 3 containing mir-218-2	congenital diaphragmatic hernia
603746	slit (Drosophila) homolog 2 containing mir-218-1	retinal ganglion cell axon guidance
603803	dachshund (Drosophila) homolog containing hypothetical miRNA-083	cell proliferation during mammalian retinogenesis and pituitary development
605317	forkhead box P2/hypothetical miRNA 169	autism & speech-language disorder-1, 602081
605547	follistatin-like 1 containing mir-198	systemic rheumatic diseases
605575	SMC4 (structural maintenance of chromosomes 4, yeast)-like 1 containing mir-16-3 and mir-15b	cell proliferation
605766	deleted in lymphocytic leukemia, 2 containing mir-16-1 and mir-15a-1	B-cell chronic lymphocytic leukemia
606157	hypothetical protein FLJ11729 containing mir-103-2	Neurodegeneration, pantothenate kinase-associated, 234200 (3);
606160	pantothenate kinase containing mir-107	pantothenate kinase-associated neurodegeneration
606161	hypothetical protein FLJ12899 containing mir-103-1	pantothenate kinase-associated neurodegeneration

From these data, it was observed that several miRNAs are predicted to be associated with human disease states. For example, several studies of autistic disorder have demonstrated linkage to a similar region of 7q (the AUTS1 locus), leading to the proposal that a single genetic factor on 7q31 contributes to both autism and language disorders, and it has been reported that the FOXP2 gene, located on human 7q31, encoding a transcription factor containing a polyglutamine tract and a forkhead domain, is mutated in a severe monogenic form of speech and language impairment, segregating within a single large pedigree, and is also disrupted by a translocation. In one recent study, association and mutation screening analysis of the FOXP2 gene was performed to assess the impact of this gene on complex language impairments and autism, and it was concluded that coding-region variants in FOXP2 do not underlie the AUTS1 linkage and that the gene is unlikely to play a role in autism or more common forms of language impairment (Newbury, et al., *Am. J. Hum. Genet.* **2002**, *70*,1318-27). However, hypothetical mir-169 is also encoded by this same genetic locus, and it is possible that mutations affecting the hypothetical mir-169 miRNA could underlie the AUTS1 linkage and play a role in language

impairment. To this end, oligomeric compounds targeting or mimicking the mir-169 miRNA may prove useful in the study, diagnosis, treatment or amelioration of this disease.

Example 30: Effects of oligomeric compounds targeting miRNAs on insulin signaling and hallmark gene expression in HepG2 cells.

Additional oligomeric compounds were screened in the assays described in Example 18. As stated above, insulin inhibits the expression of IGFBP-1, PEPCK-c and follistatin mRNAs.

Protocols for treatment of HepG2 cells and transfection of oligomeric compounds are as described in Example 18. Also as described in Example 18, forty-four hours post-transfection, the cells in the transfected wells were treated with either no insulin ("basal" Experiment 3 (below), for identification of insulin-mimetic compounds) or with 1nM insulin ("insulin treated" Experiment 4 (below), for identification of insulin sensitizers) for four hours. At the same time, in both plates, cells in some of the un-transfected control wells are treated with 100nM insulin to determine maximal insulin response. At the end of the insulin or no-insulin treatment (forty-eight hours post-transfection), total RNA is isolated from both the basal and insulin treated (1nM) 96-well plates, and the amount of total RNA from each sample is determined using a Ribogreen assay (Molecular Probes, Eugene, OR). Real-time PCR is performed on all the total RNA samples using primer/probe sets for three insulin responsive genes: PEPCK-c, IGFBP-1 and follistatin. Expression levels for each gene are normalized to total RNA, and values ± standard deviation are expressed relative to the transfectant only and negative control oligonucleotides. The compound ISIS Number 186515 (AGGTAGCTTTGATTATGTAA; SEQ ID NO: 939) is targeted to IGFBP-1 and is a phosphorothioate 5-10-5 MOE gapmer where all cytosines are 5-methylcytosines, as is used as a transfection control. The oligomeric compound ISIS Number 340341 (TAGCTTATCAGACTGATGTTGA; SEQ ID NO: 236) is a uniform 2'-MOE phosphorothioate compound targeted to mir-104 (Mourelatos), ISIS 340362 (GACTGTTGAATCTCATGGCA; SEQ ID NO: 937) is a 5-10-5 gapmer compound also targeted to mir-104 (Mourelatos), and ISIS Number 341813 (AGACACGTGCACTGTAGA; SEQ ID NO: 938) is a uniform 2'-MOE phosphorothioate compound targeted to mir-139. Results of these experiments are shown in Tables 53 and 54.

Table 53

Experiment 3: Effects of oligomeric compounds targeting miRNAs on insulin-repressed gene expression in HepG2 cells

ISIS NO:	SEQ ID NO	Pri-miRNA	IGFBP-1 (% UTC)	PEPCK-c (% UTC)	Follistatin (% UTC)
UTC	N/A	N/A	100	100	100

29848 n-mer	737	N/A	104	100	90
186515	939	IGFBP-1	193	70	67
328384	493	hypothetical miRNA-039	139	142	110
328677	586	hypothetical miRNA-120	208	145	130
328685	594	mir-219	157	219	100
328691	600	mir-145	105	108	93
328759	668	mir-216	356	98	266
328761	670	hypothetical miRNA-138	118	48	91
328765	674	mir-215	88	93	87
328773	682	mir-15a-2	148	138	131
328779	688	hypothetical mir-177	135	123	109
340341	236	mir-104 (Mourelatos)	110	129	94
340362	937	mir-104 (Mourelatos)	157	168	123
341813	938	mir-139	137	121	100

Under "basal" conditions (without insulin), treatments of HepG2 cells with oligomeric compounds of the present invention resulting in decreased mRNA expression levels of the PEPCK-c, IGFBP-1 and/or follistatin marker genes indicate that the oligomeric compounds have an insulin mimetic effect. Treatments with oligomeric compounds of the present invention resulting in an increase in mRNA expression levels of the PEPCK-c, IGFBP-1 and/or follistatin marker genes indicate that these compounds inhibit or counteract the normal insulin repression of mRNA expression of these genes.

From these data, it is evident that the oligomeric compound ISIS Number 328761 targeting hypothetical mir-138, for example, results in a 52% decrease in PEPCK-c mRNA, a marker widely considered to be insulin-responsive. Thus, this oligomeric compound may be useful as a pharmaceutical agent with insulin mimetic properties in the treatment, amelioration, or prevention of diabetes or other metabolic diseases.

Table 54

Experiment 4: Effects of oligomeric compounds targeting miRNAs on insulin-sensitization of gene expression in HepG2 cells

ISIS NO:	SEQ ID NO	Pri-miRNA	IGFBP-1 (% UTC)	PEPCK-c (% UTC)	Follistatin (% UTC)
UTC(1 nm insulin)	N/A	N/A	100	100	100
29848 n-mer	737	N/A	92	90	95
186515	939	IGFBP-1	105	40	39
328384	493	hypothetical miRNA-039	102	114	121
328677	586	hypothetical miRNA-120	159	117	118
328685	594	mir-219	143	184	157
328691	600	mir-145	101	97	104
328759	668	mir-216	212	92	224
328761	670	hypothetical miRNA-138	93	55	98
328765	674	mir-215	94	73	97
328773	682	mir-15a-2	136	93	148
328779	688	hypothetical mir-177	128	78	119

340341	236	mir-104 (Mourelatos)	113	115	120
340362	937	mir-104 (Mourelatos)	129	104	119
341813	938	mir-139	117	88	102

In HepG2 cells treated with 1nM insulin, treatments with oligomeric compounds of the present invention resulting in a decrease in mRNA expression levels of the PEPCK-c, IGFBP-1 and/or follistatin marker genes indicate that these compounds have an insulin sensitization effect. Treatments with oligomeric compounds of the present invention resulting in an increase in mRNA expression levels of the PEPCK-c, IGFBP-1 and/or follistatin marker genes indicate that these compounds inhibit or counteract the normal insulin response of repression of mRNA expression of these genes.

From these data, it is evident that the oligomeric compounds, ISIS Number 328761 targeting hypothetical mir-138 and ISIS Number 328765 targeting mir-215, for example, were observed to result in a 45% and a 27% reduction, respectively, of PEPCK-c mRNA expression, widely considered to be a marker of insulin-responsiveness. Furthermore, mRNA levels of the IGFBP-1 and follistatin genes were also reduced. Thus, these oligomeric compounds may be useful as pharmaceutical agents with insulin-sensitizing properties in the treatment, amelioration, or prevention of diabetes or other metabolic diseases.

Example 31: Adipocyte assay of oligomeric compounds

The effect of several oligomeric compounds of the present invention targeting miRNA target nucleic acids on the expression of markers of cellular differentiation was examined in differentiating adipocytes.

As described in Example 13, some genes known to be upregulated during adipocyte differentiation include HSL, aP2, Glut4 and PPARγ. These genes play important roles in the uptake of glucose and the metabolism and utilization of fats. An increase in triglyceride content is another well-established marker for adipocyte differentiation.

For assaying adipocyte differentiation, expression of the four hallmark genes, HSL, aP2, Glut4, and PPARγ, as well as triglyceride (TG) accumulation were measured as previously described in adipocytes transfected with uniform 2'-MOE or chimeric gapmer phosphorothioate (PS) oligomeric compounds. Triglyceride levels as well as mRNA levels for each of the four adipocyte differentiation hallmark genes are expressed as a percentage of untreated control (UTC) levels. Results are shown in Table 55.

Table 55

Effects of oligomeric compounds targeting miRNAs on expression of adipocyte differentiation markers

ISIS Number	SEQ ID NO	TG	HSL	AP2	Glut4	PPAR gamma
UTC	N/A	100	100	100	100	100
ISIS-29848 n-mer	737	89	84	89	96	100
327877	295	109	82	77	119	85
327888	306	132	134	102	84	103
327904	322	56	42	65	40	54
327909	327	132	130	88	132	96
327927	345	125	120	114	120	108
327928	346	45	52	77	39	57
327933	351	127	132	82	127	100
327937	355	81	77	76	63	92
327951	369	76	100	91	81	84
327953	371	94	94	92	112	90
327956	374	80	90	102	69	91
327960	378	47	52	52	34	76
328093	395	59	89	97	73	99
328112	414	92	89	73	97	79
328114	416	110	134	123	116	106
328132	434	120	89	81	67	94
328340	449	76	130	85	112	110
328362	471	73	83	59	80	78
328400	509	60	40	34	18	67
328417	526	83	98	87	68	94
328434	543	91	96	85	83	79
328651	560	93	109	84	78	106
328677	586	34	68	61	44	89
328685	594	50	100	73	69	91
328691	600	130	156	166	144	105
328759	668	87	105	108	66	95

For these data, values for triglyceride accumulation above 100 are considered to indicate that the compound has the ability to stimulate triglyceride accumulation, whereas values at or below 100 indicate that the compound inhibits triglyceride accumulation. With respect to leptin secretion, values above 100 are considered to indicate that the compound has the ability to stimulate secretion of the leptin hormone, and values at or below 100 indicate that the compound has the ability to inhibit secretion of leptin. With respect to the four adipocyte differentiation hallmark genes, values above 100 are considered to indicate induction of cell differentiation, whereas values at or below 100 indicate that the compound inhibits differentiation.

Several compounds were found to have remarkable effects. For example, the oligomeric compounds ISIS Number 327904 (SEQ ID NO: 322), targeted to mir-181a-1, ISIS Number 327928 (SEQ ID NO: 346), targeted to mir-29a, ISIS Number 327960 (SEQ ID NO: 378), targeted to mir-215, ISIS Number 328400 (SEQ ID NO: 509), targeted to mir-196-2, and ISIS Number 328677 (SEQ ID NO: 586), targeted to hypothetical miRNA-120 were shown to reduce

the expression levels of all five markers of adipocyte differentiation, indicating that these oligomeric compounds have the ability to block adipocyte differentiation. Therefore, these oligomeric compounds may be useful as therapeutic agents with applications in the treatment, attenuation or prevention of obesity, hyperlipidemia, atherosclerosis, atherogenesis, diabetes, hypertension, or other metabolic diseases as well as in the maintenance of the pluripotent phenotype of stem or precursor cells.

The oligomeric compounds ISIS Number 328691 (SEQ ID NO: 600) targeted to mir-145, ISIS Number 328114 (SEQ ID NO: 416) targeted to hypothetical miRNA-138, and ISIS Number 327927 (SEQ ID NO: 345) targeted to mir-15b are examples of compounds which exhibit an increase in all five markers of adipocyte differentiation. Additionally, the oligomeric compound ISIS Number 327909 (SEQ ID NO: 327) targeted to mir-196-2 exhibited an increase in three of the five markers of adipocyte differentiation. Thus, these oligomeric compounds may be useful as pharmaceutical agents in the treatment of diseases in which the induction of adipocyte differentiation is desirable, such as anorexia, or for conditions or injuries in which the induction of cellular differentiation is desireable, such as Alzheimers disease or central nervous system injury, in which regeneration of neural tissue would be beneficial. Furthermore, these oligomeric compounds may be useful in the treatment, attenuation or prevention of diseases in which it is desireable to induce cellular differentiation and/or quiescence, for example in the treatment of hyperproliferative disorders such as cancer.

Example 32: Effects of oligomeric compounds on endothelial tube formation assay

Angiogenesis is the growth of new blood vessels (veins and arteries) by endothelial cells. This process is important in the development of a number of human diseases, and is believed to be particularly important in regulating the growth of solid tumors. Without new vessel formation it is believed that tumors will not grow beyond a few millimeters in size. In addition to their use as anti-cancer agents, inhibitors of angiogenesis have potential for the treatment of diabetic retinopathy, cardiovascular disease, rheumatoid arthritis and psoriasis (Carmeliet and Jain, *Nature,* **2000**, *407*, 249-257; Freedman and Isner, *J. Mol. Cell. Cardiol.,* **2001**, *33*, 379-393; Jackson et al., *Faseb J.,* **1997**, *11*, 457-465; Saaristo et al., *Oncogene,* **2000**, *19*, 6122-6129; Weber and De Bandt, *Joint Bone Spine,* **2000**, *67*, 366-383; Yoshida et al., *Histol. Histopathol.,* **1999**, *14*, 1287-1294).

Endothelial tube formation assay as a measure of angiogenesis:

Angiogenesis is stimulated by numerous factors that promote interaction of endothelial cells with each other and with extracellular matrix molecules, resulting in the formation of

capillary tubes. This morphogenic process is necessary for the delivery of oxygen to nearby tissues and plays an essential role in embryonic development, wound healing, and tumor growth (Carmeliet and Jain, *Nature,* **2000**, *407*, 249-257). Moreover, this process can be reproduced in a tissue culture assay that evaluates the formation of tube-like structures by endothelial cells. There are several different variations of the assay that use different matrices, such as collagen I (Kanayasu et al., *Lipids,* **1991**, *26*, 271-276), Matrigel (Yamagishi et al., *J. Biol. Chem.,* **1997**, *272*, 8723-8730) and fibrin (Bach et al., *Exp. Cell Res.,* **1998**, *238*, 324-334), as growth substrates for the cells. In this assay, human umbilical vein endothelial cells (HuVECs) are plated on a matrix derived from the Engelbreth-Holm-Swarm mouse tumor, which is very similar to Matrigel (Kleinman et al., *Biochemistry,* **1986**, *25*, 312-318; Madri and Pratt, *J. Histochem. Cytochem.,* **1986**, *34*, 85-91). Untreated HuVECs form tube-like structures when grown on this substrate. Loss of tube formation *in vitro* has been correlated with the inhibition of angiogenesis *in vivo* (Carmeliet and Jain, *Nature,* **2000**, *407*, 249-257; Zhang et al., *Cancer Res.*, **2002**, *62*, 2034-2042), which supports the use of *in vitro* tube formation as an endpoint for angiogenesis.

In one embodiment, primary human umbilical vein endothelial cells (HuVECs) were used to measure the effects of oligomeric compounds targeted to miRNAs on tube formation activity. HuVECs were routinely cultured in EBM (Clonetics Corporation, Walkersville, MD) supplemented with SingleQuots supplements (Clonetics Corporation, Walkersville, MD). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence and were maintained for up to 15 passages. HuVECs are plated at 3000 cells/well in 96-well plates. One day later, cells are transfected with oligomeric compounds. The tube formation assay is performed using an *in vitro* Angiogenesis Assay Kit (Chemicon International, Temecula, CA).

A scrambled control compound, ISIS 29848 (NNNNNNNNNNNNNNNNNNNN; where N is A,T,C or G; herein incorporated as SEQ ID NO: 737) served as a negative control. ISIS 196103 (AGCCCATTGCTGGACATGCA; incorporated herein as SEQ ID NO: 940) targets integrin beta 3 and was used as a positive control to inhibit endothelial tube formation. ISIS 29248 and ISIS 196103 are chimeric 5-10-5 2'-MOE gapmer oligonucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotides. All cytidine residues are 5-methylcytidines. ISIS 342672 (SEQ ID NO: 789) contains 13 mismatches with respect to the mature mir-143 miRNA, and was also used as a negative control. ISIS 342672 is a uniform 2'-MOE phosphorothioate oligomeric compound 22 nucleotides in length. All cytidine residues are 5-methylcytidines.

Oligomeric compound was mixed with LIPOFECTIN™ (Invitrogen Life Technologies,

Carlsbad, CA) in OPTI-MEM™ (Invitrogen Life Technologies, Carlsbad, CA) to acheive a final concentration of 75 nM of oligomeric compound and 2.25 μg/mL LIPOFECTIN™. Before adding to cells, the oligomeric compound, LIPOFECTIN™ and OPTI-MEM™ were mixed thoroughly and incubated for 0.5 hrs. Untreated control cells received LIPOFECTIN™ only. The medium was removed from the plates and the plates were tapped on sterile gauze. Each well was washed in 150 μl of phosphate-buffered saline. The wash buffer in each well was replaced with 100 μL of the oligomeric compound/OPTI-MEM™/LIPOFECTIN™ cocktail. Compounds targeted to miRNAs were tested in triplicate, and ISIS 29848 was tested in up to six replicates. The plates were incubated for 4 hours at 37° C, after which the medium was removed and the plate was tapped on sterile gauze. 100 μl of full growth medium was added to each well. Fifty hours after transfection, cells are transferred to 96-well plates coated with ECMa-trix™ (Chemicon Inter-national). Under these conditions, untreated HuVECs form tube-like structures. After an overnight incubation at 37° C, treated and untreated cells are inspected by light microscopy. Individual wells are assigned discrete scores from 1 to 5 depending on the extent of tube formation. A score of 1 refers to a well with no tube formation while a score of 5 is given to wells where all cells are forming an extensive tubular network. Results are expressed as a percentage of the level of the tube formation observed in cultures not treated with oligonucleotide, and are shown in Tables 56-59.

Table 56

Effect of compounds targeting miRNAs on Tube Formation Activity in HuVECs

ISIS NO:	SEQ ID NO:	Pri-miRNA	% Activity Relative to UTC
UTC	N/A	N/A	100
196103 positive control	940	Integrin beta 3	35.7
342672 negative control	789	N/A	46.4
327873	291	mir-140	100.0
327875	293	mir-34	71.4
327876	294	mir-29b-1	50.0
327877	295	mir-16-3	78.6
327878	296	mir-203	57.1
327879	297	mir-7-1	71.4
327880	298	mir-10b	57.1
327881	299	mir-128a	50.0
327882	300	mir-153-1	107.1
327883	301	mir-27b	92.9
327884	302	mir-96	78.6
327885	303	mir-17as/mir-91	50.0
327886	304	mir-123/mir-126as	42.9
327887	305	mir-132	57.1
327888	306	mir-108-1	100.0
327889	307	mir-23b	50.0
327890	308	let-7i	92.9

327891	309	mir-212	50.0
327892	310	mir-131-2/mir-9	57.1
327893	311	let-7b	100.0
327894	312	mir-1d	100.0
327895	313	mir-122a	100.0
327896	314	mir-22	64.3
327898	316	mir-142	100.0

From these data, it was observed that ISIS Number 327886 targeted to mir-123/mir126as suppressed tube formation, indicating that this compound may be useful as an angiogenesis inhibitor and/or anti-tumor agent, with potential therapeutic applications in the treatment of diabetic retinopathy, cardiovascular disease, rheumatoid arthritis, psoriasis, as well as cancer.

Table 57

Effect of compounds targeting miRNAs on Tube Formation Activity in HuVECs

ISIS NO:	SEQ ID NO:	Pri-miRNA	% Activity Relative to UTC
UTC	N/A	N/A	100
196103 positive control	940	Integrin beta 3	24.1
342672 negative control	789	N/A	58.6
327899	317	mir-183	34.5
327900	318	mir-214	55.2
327901	319	mir-143	48.3
327902	320	mir-192-1	41.4
327903	321	let-7a-3	103.5
327904	322	mir-181a	89.7
327905	323	mir-205	48.3
327906	324	mir-103-1	69.0
327907	325	mir-26a	62.1
327908	326	mir-33a	103.5
327909	327	mir-196-2	96.6
327910	328	mir-107	55.2
327911	329	mir-106	75.9
327913	331	mir-29c	69.0
327914	332	mir-130a	82.8
327915	333	mir-218-1	69.0
327916	334	mir-124a-2	96.6
327917	335	mir-21	82.8
327918	336	mir-144	96.6
327919	337	mir-221	103.5
327920	338	mir-222	41.4
327921	339	mir-30d	96.6
327922	340	mir-19b-2	89.7
327923	341	mir-128b	48.3

From these data, it was observed that ISIS Number 327899 targeted to mir-183, ISIS Number 327902 targeted to mir-192-1, and ISIS Number 327920 targeted to mir-222 suppressed tube formation, indicating that these compounds may be useful as an angiogenesis inhibitors

and/or anti-tumor agents, with potential therapeutic applications in the treatment of diabetic retinopathy, cardiovascular disease, rheumatoid arthritis, psoriasis, as well as cancer.

Table 58

Effect of compounds targeting miRNAs on Tube Formation Activity in HuVECs

ISIS NO:	SEQ ID NO:	Pri-miRNA	% Activity Relative to UTC
UTC	N/A	N/A	100
196103 positive control	940	Integrin beta 3	29.6
342672 negative control	789	N/A	55.6
327924	342	mir-129-2	88.9
327925	343	mir-133b	44.4
327926	344	let-7d	96.3
327927	345	mir-15b	59.3
327928	346	mir-29a-1	37.0
327929	347	mir-199b	51.9
327930	348	let-7e	88.9
327931	349	let-7c	103.7
327932	350	mir-204	51.9
327933	351	mir-145	59.3
327934	352	mir-213/mir-181a	51.9
327935	353	mir-20	74.1
327936	354	mir-133a-1	51.9
327937	355	mir-138-2	88.9
327938	356	mir-98	96.3
327939	357	mir-125b-1	66.7
327940	358	mir-199a-2	59.3
327941	359	mir-181b	74.1
327942	360	mir-141	74.1
327943	361	mir-18	81.5
327944	362	mir-220	37.0
327945	363	mir-24-2	59.3
327946	364	mir-211	51.9
327947	365	mir-101-3	81.5

From these data, it was observed that ISIS Number 327925 targeted to mir-133b, ISIS Number 327928 targeted to mir-29a-1, and ISIS Number 327944 targeted to mir-220 suppressed tube formation, indicating that these compounds may be useful as an angiogenesis inhibitors and/or anti-tumor agents, with potential therapeutic applications in the treatment of diabetic retinopathy, cardiovascular disease, rheumatoid arthritis, psoriasis, as well as cancer.

Table 59

Effect of compounds targeting miRNAs on Tube Formation Activity in HuVECs

ISIS Number	SEQ ID NO:	Pri-miRNA	% Activity Relative to UTC
UTC	N/A	N/A	100
196103 positive control	940	Integrin beta 3	26.7
342672 negative control	789	N/A	60.0
327874	292	mir-30a	46.7

327897	315	mir-92-1	40.0
327901	319	mir-143	100.0
327948	366	mir-30b	33.3
327949	367	mir-10a	66.7
327950	368	mir-19a	73.3
327951	369	mir-15a-1	73.3
327952	370	mir-137	53.3
327953	371	mir-219	53.3
327954	372	mir-148b	53.3
327955	373	mir-130b	46.7
327956	374	mir-216	46.7
327957	375	mir-100-1	66.7
327958	376	mir-187	40.0
327959	377	mir-210	40.0
327960	378	mir-215	53.3
327961	379	mir-223	53.3
327962	380	mir-30c	53.3
327963	381	mir-26b	93.3
327964	382	mir-152	86.7
327965	383	mir-135-1	100.0
327966	384	mir-217	40.0
327967	385	let-7g	93.3
327968	386	mir-33b	93.3

From these data, it was observed that ISIS Number 327948 targeted to mir-30b, ISIS Number 327958 targeted to mir-187, ISIS Number 327959 targeted to mir-210, and ISIS Number 327966 targeted to mir-217 suppressed tube formation, indicating that these compounds may be useful as an angiogenesis inhibitors and/or anti-tumor agents, with potential therapeutic applications in the treatment of diabetic retinopathy, cardiovascular disease, rheumatoid arthritis, psoriasis, as well as cancer.

Example 33: Effect of oligomeric compounds on miRNA target protein expression

Several mRNA transcripts have been predicted to be regulated by miRNAs (Lewis et al., *Cell,* **2003**, *115*, 787-798). For example, the mRNAs encoded by six genes, 1) inwardly rectifying potassium channel Kir2.2 (GenBank Accession AB074970, SEQ ID NO: 872); 2) synaptotagmin III (GenBank Accession BC028379, SEQ ID NO: 873); 3) mitogen-activated protein kinase 7/extracellular signal-regulated kinase 5 (ERK5) (GenBank Accession NM_139032.1, SEQ ID NO: 861); 4) protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform (PPP2CB) (GenBank Accession NM_004156.1, SEQ ID NO: 814); 5) glyoxalase I (GenBank Accession NM_006708.1, SEQ ID NO: 821); and 6) LIM domain only 4 (LMO4) (GenBank Accession NM_006769.2, SEQ ID NO: 865), are believed to have mir-143 binding sites within their 3'-UTRs. The latter three genes encode mRNAs that were identified as potential targets of mir-143 by the RACE-PCR experiments described, *supra.* Thus, the mir-143 miRNA is predicted to regulate some or all of these genes.

When miRNAs have effects on the expression of downstream genes or proteins encoded by these genes, it is advantageous to measure the protein levels of those gene products, and to do this, western blot (immunoblot) analysis is employed. Immunoblot analysis is carried out using standard methods. Briefly, preadipocytes and differentiating adipocytes were cultured as described previously, and differentiating adipocytes are sampled at several timepoints after stimulation of differentiation. Cells were treated with 250 nM oligomeric compounds and harvested 16-20 h after oligomeric compound treatment. Cells were washed, lysed in RIPA buffer with protease inhibitor cocktail (Roche Diagnostics Corporation, Indianapolis, IN), suspended in Laemmli buffer (20 ul/well), boiled for 5 minutes and loaded onto either an 8% SDS-PAGE or a 4-20% gradient SDS-PAGE gel. Gels are run for approximately 1.5 hours at 150 V, and transferred to PVDF membrane for western blotting. Appropriate primary antibody directed to a target is used, with a radiolabeled or fluorescently labeled secondary antibody directed against the primary antibody species. Because expression levels of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein remain constant, an antibody recognizing the GAPDH protein (Abcam, Cambridge, MA) can be used in a re-probing of the membrane to verify equal protein loading. It is also understood that antisense oligomeric compounds specifically targeting and known to inhibit the expression of the mRNA and protein endproducts of the gene of interest can be used as controls in these experiments. Bands are visualized and quantitated using a PHOSPHORIMAGER™ (Molecular Dynamics, Sunnyvale CA) or the ChemiDoc™ system (Bio-Rad Laboratories, Inc., Hercules, California). Thus, the effects of treatment of many cell types (including, but not limited to, preadipocytes, differentiating adipocytes, HeLa, T-24 and A549 cells) with oligomeric compounds of the present invention on the levels of gene expression products can be assessed. It is understood that one of ordinary skill in the art can use immunoblot analysis to examine the expression of any protein predicted to be the downstream expression product of a target of a miRNA. Similarly, using methods described above, real-time RT-PCR methods can also be used to examine the mRNA expression levels of any of these predicted targets of the mir-143 miRNA. More specifically, immunoblot analysis and/or real-time RT-PCR methods can be used to examine the effects of treatment with oligomeric compounds on the protein or mRNA levels, respectively, produced by the Kir2.2, synaptotagmin III, ERK5, PPP2CB, glyoxalase I, and/or LMO4 genes in a variety of cell types.

In one embodiment of the invention, immunoblot analysis was used to assess the effects of the oligomeric compound, ISIS Number 327901 (SEQ ID NO: 319) targeting mir-143, on expression levels of the PPP2CB protein in differentiating adipocytes. It was observed that, upon treatment with ISIS 327901, PPP2CB protein levels were higher in differentiating adipocytes

both 7- and 10-days post-differentiation than in pre-adipocytes or in untreated differentiating adipocytes from the same timepoints. Thus, mir-143 appears to negatively regulate the expression of the PPP2CB gene, presumably by inhibiting translation of the PPP2CB mRNA into protein, and upon treatment with the oligomeric compound ISIS 327901, this inhibition of PPP2CB protein expression was relieved.

In one embodiment of the invention, immunoblot analysis was used to assess the effects of the oligomeric compound, ISIS Number 327901 (SEQ ID NO: 319) targeting mir-143, on expression levels of the ERK5 protein in differentiating adipocytes. It was observed that, upon treatment of cells with ISIS 327901, ERK5 protein levels were approximately 2- 2.5-fold higher in differentiating adipocytes both 7- and 10-days post-differentiation than in pre-adipocytes or in untreated differentiating adipocytes from the same timepoints. Thus, mir-143 appears to negatively regulate the expression of the ERK5 gene presumably by inhibiting translation of the ERK5 mRNA into protein, either directly (by mir-143 binding an ERK5 *cis*-regulatory sequence) or indirectly (by mir-143 regulating another target gene that regulates ERK5); upon treatment with the oligomeric compound ISIS 327901, this mir-143-dependent inhibition of ERK5 expression was relieved. It is known that ERK5 promotes cell growth and proliferation in response to tyrosine kinase signaling. In light of the involvement of mir-143 in adipocyte differentiation disclosed in several examples in the present invention, as well as the role of mir-143 in regulating ERK5, it is predicted that ERK5 and mir-143 are together involved regulating the balance between cellular proliferation and differentiation.

It is understood that the oligomeric compounds of the present invention, including miRNA mimics, can also be tested for their effects on the expression of the protein endproducts of targets of miRNAs. For example, an oligomeric compound such as a mir-143 mimic can be used to treat differentiating adipocytes, and is predicted to result in a reduction of Kir2.2, synaptotagmin III, ERK5, PPP2CB, glyoxalase I, and/or LMO4 protein expression levels.

The phosphatase and tensin homolog (mutated in multiple advanced cancers 1) (PTEN) tumor suppressor mRNA (GenBank Accession NM_000314, incorporated herein as SEQ ID NO: 941) has been predicted to be a potential target of the mir-19a miRNA (Lewis et al., *Cell,* **2003**, *115*, 787-798). Oligomeric compounds that target or mimic the mir-19a miRNA or mir-19a pri-miRNA can be used to treat cells and, using the methods described above, the effects of these oligomeric compounds on the expression of the PTEN protein and mRNA levels can be assessed. It is predicted that the mir-19a miRNA, or an oligomeric compound acting as a mir-19a mimic, would inhibit expression of the PTEN tumor suppressor mRNA and protein, and that treatment with oligomeric compounds targeting mir-19a would reverse this inhibition. It is also understood

that other antisense oligomeric compounds specifically targeting and known to inhibit the expression of the mRNA and protein endproducts of the gene interest can be used as controls in these experiments.

Example 34: Additional oligomeric compounds targeting miRNAs

In accordance with the present invention, oligomeric compounds were designed and synthesized to target or mimic one or more miRNA genes or gene products. Pri-miRNAs, pre-miRNAs and mature miRNAs represent target nucleic acids to which the oligomeric compounds of the present invention were designed and synthesized. Oligomeric compounds of the present invention can also be designed and synthesized to mimic the pri-miRNA, pre-miRNA or mature miRNA structure while incorporating certain chemical modifications that alter one or more properties of the mimic, thus creating a construct with superior properties as compared to the endogenous precursor or mature miRNA.

In accordance with the present invention, oligomeric compounds were designed to target or mimic one or more human, mouse, rat, or *Drosophila* pri-miRNAs, pre-miRNAs or mature miRNAs.

A list of human pri-miRNAs and the mature miRNAs predicted to derive from them is shown in Table 60. "Pri-miRNA name" indicates the gene name for each of the pri-miRNAs, and "pri-miRNA sequence" indicates the sequence of the predicted primary miRNA transcript. Also given in table 60 are the name and sequence of the mature miRNA derived from the pri-miRNA. Mature miRNA sequences from pri-miRNA precursors have been proposed by several groups; consequently, for a given pri-miRNA sequence, several miRNAs may be disclosed and given unique names, and thus a given pri-miRNA sequence may occur repeatedly in the table. The sequences are written in the 5' to 3' direction and are represented in the DNA form. It is understood that a person having ordinary skill in the art would be able to convert the sequence of the targets to their RNA form by simply replacing the thymidine (T) with uracil (U) in the sequence.

Table 60

Human pri-miRNA sequences and the corresponding mature miRNAs

Pri-miRNA name	Pri-miRNA sequence	SEQ ID NO	Mature miRNA name	Mature miRNA sequence	SEQ ID NO
mir-27b	TGACCTCTCTAACAAG GTGCAGAGCTTAGCTG ATTGGTGAACAGTGAT TGGTTTCCGCTTTGTT CACAGTGGCTAAGTTC	17	mir-27b	TTCACAGTGGCTAAGTTCTG	202

	TGCACCTGAAGAGAAG GTGAGATGGGGACA				
mir-27b	TGACCTCTCTAACAAG GTGCAGAGCTTAGCTG ATTGGTGAACAGTGAT TGGTTTCCGCTTTGTT CACAGTGGCTAAGTTC TGCACCTGAAGAGAAG GTGAGATGGGGACA	17	miR-27* (Michael et al)	TTCACAGTGGCTAAGTTCTGC	1059
mir-23b	GCGCTGCTCTCAGGTG CTCTGGCTGCTTGGGT TCCTGGCATGCTGATT TGTGACTTAAGATTAA AATCACATTGCCAGGG ATTACCACGCAACCAC GACCTTGGCTGCTC	23	mir-23b	ATCACATTGCCAGGGATTACCAC	208
glutamate receptor, ionotrophic, AMPA 3/ hypothetical miRNA-033	TGGTGTGGCAACCCCT AAAGGCTCAGCATTAA GGTGGGTGGAATAATA TAACAATATCCGTGTT GTTATAGTATTCCACC TACCCTGATGCATTTT GTTGTCATTTTCTT	36	hypothetical miRNA-033	TGTTATAGTATTCCACCTACC	1060
LOC 114614 containing miR-155/ hypothetical miRNA-071	GCTTGCTGTAGGCTGT ATGCTGTTAATGCTAA TCGTGATAGGGGTTTT TGCCTCCAACTGACTC CTACATATTAGCATTA ACAGTGTATGATGCCT GTTACTAGCATTCA	74	hypothetical miRNA-071	TGCTAATCGTGATAGGGGTTT	1061
LOC 114614 containing miR-155/ hypothetical miRNA-071	GCTTGCTGTAGGCTGT ATGCTGTTAATGCTAA TCGTGATAGGGGTTTT TGCCTCCAACTGACTC CTACATATTAGCATTA ACAGTGTATGATGCCT GTTACTAGCATTCA	74	mir-155 (RFAM)	TTAATGCTAATCGTGATAGGGG	1062
collagen, type I, alpha 1/ hypothetical miRNA-144	CACGCATGAGCGGACG CTAACCCCCTCCCCAG CCACAAAGAGTCTACA TGTCTAGGGTCTAGAC ATGTTCAGCTTTGTGG ACCTCCGGCTCCTGCT CCTCTTAGCGGCCA	147	hypothetical miRNA-144	AGACATGTTCAGCTTTGTGGA	1063
sterol regulatory element-binding protein-1/ mir-33b	GGGGGCCGAGAGAGGC GGGCGGCCCCGCGGTG CATTGCTGTTGCATTG CACGTGTGTGAGGCGG GTGCAGTGCCTCGGCA GTGCAGCCCGGAGCCG GCCCCTGGCACCAC	168	mir-33b	GTGCATTGCTGTTGCATTG	286
tight junction protein 1 (zona occludens 1)/ hypothetical miRNA-183	ACTCCAGGTGAAACAC TGCTGAGTCCTTTGGT GATGTGTGGTCCCCAT GGCCTCAAGTTCCTGA AGCCTGTGGAGCTGCG CTTACCACACTGTGCG TCCATGACTCCTGA	186	hypothetical miRNA-183	AGCCTGTGGAGCTGCGCTTAC	1064

mir-140	CTGTGTGTGTCTCTCT CTGTGTCCTGCCAGTG GTTTTACCCTATGGTA GGTTACGTCATGCTGT TCTACCACAGGGTAGA ACCACGGACAGGATAC CGGGGCACCCTCTG	4	mir-140	AGTGGTTTTACCCTATGGTAG	192
mir-140	CTGTGTGTGTCTCTCT CTGTGTCCTGCCAGTG GTTTTACCCTATGGTA GGTTACGTCATGCTGT TCTACCACAGGGTAGA ACCACGGACAGGATAC CGGGGCACCCTCTG	4	miR-140-as	TACCACAGGGTAGAACCACGGA	1065
mir-140	CTGTGTGTGTCTCTCT CTGTGTCCTGCCAGTG GTTTTACCCTATGGTA GGTTACGTCATGCTGT TCTACCACAGGGTAGA ACCACGGACAGGATAC CGGGGCACCCTCTG	4	mir-239* (Kosik)	TACCACAGGGTAGAACCACGGACA	1066
mir-34	GGCCAGCTGTGAGTGT TTCTTTGGCAGTGTCT TAGCTGGTTGTTGTGA GCAATAGTAAGGAAGC AATCAGCAAGTATACT GCCCTAGAAGTGCTGC ACGTTGTGGGGCCC	6	mir-34	TGGCAGTGTCTTAGCTGGTTGT	194
mir-34	GGCCAGCTGTGAGTGT TTCTTTGGCAGTGTCT TAGCTGGTTGTTGTGA GCAATAGTAAGGAAGC AATCAGCAAGTATACT GCCCTAGAAGTGCTGC ACGTTGTGGGGCCC	6	miR-172 (RFAM-M. mu.)	TGGCAGTGTCTTAGCTGGTTGTT	1067
mir-203	GTGTTGGGGACTCGCG CGCTGGGTCCAGTGGT TCTTAACAGTTCAACA GTTCTGTAGCGCAATT GTGAAATGTTTAGGAC CACTAGACCCGGCGGG CGCGGCGACAGCGA	10	mir-203	GTGAAATGTTTAGGACCACTAG	197
mir-203	GTGTTGGGGACTCGCG CGCTGGGTCCAGTGGT TCTTAACAGTTCAACA GTTCTGTAGCGCAATT GTGAAATGTTTAGGAC CACTAGACCCGGCGGG CGCGGCGACAGCGA	10	miR-203 (RFAM-M. mu.)	TGAAATGTTTAGGACCACTAG	1068
mir-203	GTGTTGGGGACTCGCG CGCTGGGTCCAGTGGT TCTTAACAGTTCAACA GTTCTGTAGCGCAATT GTGAAATGTTTAGGAC CACTAGACCCGGCGGG CGCGGCGACAGCGA	10	miR-203 (Tuschl)	TGAAATGTTTAGGACCACTAGA	1069
mir-7_1/mir-7_1*	TTGGATGTTGGCCTAG TTCTGTGTGGAAGACT AGTGATTTTGTTGTTT TTAGATAACTAAATCG ACAACAAATCACAGTC	11	mir-7_1*_Ruvkun	CAACAAATCACAGTCTGCCATA	1070

	TGCCATATGGCACAGG CCATGCCTCTACAG				
mir-7_1/mir-7_1*	TTGGATGTTGGCCTAG TTCTGTGTGGAAGACT AGTGATTTTGTTGTTT TTAGATAACTAAATCG ACAACAAATCACAGTC TGCCATATGGCACAGG CCATGCCTCTACAG	11	mir-7	TGGAAGACTAGTGATTTTGTT	198
mir-10b	CCAGAGGTTGTAACGT TGTCTATATATACCCT GTAGAACCGAATTTGT GTGGTATCCGTATAGT CACAGATTCGATTCTA GGGGAATATATGGTCG ATGCAAAAACTTCA	12	miR-10b (Tuschl)	CCCTGTAGAACCGAATTTGTGT	1071
mir-10b	CCAGAGGTTGTAACGT TGTCTATATATACCCT GTAGAACCGAATTTGT GTGGTATCCGTATAGT CACAGATTCGATTCTA GGGGAATATATGGTCG ATGCAAAAACTTCA	12	mir-10b	TACCCTGTAGAACCGAATTTGT	199
mir-10b	CCAGAGGTTGTAACGT TGTCTATATATACCCT GTAGAACCGAATTTGT GTGGTATCCGTATAGT CACAGATTCGATTCTA GGGGAATATATGGTCG ATGCAAAAACTTCA	12	miR-10b (Michael et al)	TACCCTGTAGAACCGAATTTGTG	1072
mir-128a	TGCAATAATTGGCCTT GTTCCTGAGCTGTTGG ATTCGGGGCCGTAGCA CTGTCTGAGAGGTTTA CATTTCTCACAGTGAA CCGGTCTCTTTTTCAG CTGCTTCCTGGCTT	13	mir-128 (Kosik)	TCACAGTGAACCGGTCTCTTT	1073
mir-128a	TGCAATAATTGGCCTT GTTCCTGAGCTGTTGG ATTCGGGGCCGTAGCA CTGTCTGAGAGGTTTA CATTTCTCACAGTGAA CCGGTCTCTTTTTCAG CTGCTTCCTGGCTT	13	mir-128a	TCACAGTGAACCGGTCTCTTTT	200
mir-153_1	TCTCTCTCTCCCTCAC AGCTGCCAGTGTCATT TTTGTGATCTGCAGCT AGTATTCTCACTCCAG TTGCATAGTCACAAAA GTGATCATTGGCAGGT GTGGCTGCTGCATG	14	mir-153	TTGCATAGTCACAAAAGTGA	201
mir-153_2	TGCCAGCTAATTAGCG GTGGCCAGTGTCATTT TTGTGATGTTGCAGCT AGTAATATGAGCCCAG TTGCATAGTCACAAAA GTGATCATTGGAAACT GTGACTGTACTGCA	15	mir-153	TTGCATAGTCACAAAAGTGA	201
hypothetical miR-13/miR-190	CTGGATGCCTTTTCTG CAGGCCTCTGTGTGAT ATGTTTGATATATTAG	16	hypothetical miRNA-	TATCAAACATATTCCTACAGT	1074

	GTTGTTATTTAATCCA ACTATATATCAAACAT ATTCCTACAGTGTCTT GCCCTGTCTCCGGG		013		
hypothetical miR-13/miR-190	CTGGATGCCTTTTCTG CAGGCCTCTGTGTGAT ATGTTTGATATATTAG GTTGTTATTTAATCCA ACTATATATCAAACAT ATTCCTACAGTGTCTT GCCCTGTCTCCGGG	16	miR-190	TGATATGTTTGATATATTAGGT	1075
mir-123/mir-126	GCCACGCCTCCGCTGG CGACGGGACATTATTA CTTTTGGTACGCGCTG TGACACTTCAAACTCG TACCGTGAGTAATAAT GCGCCGTCCACGGCAC CGCATCGAAAACGC	20	mir-123/mir-126as	CATTATTACTTTTGGTACGCG	205
mir-123/mir-126	GCCACGCCTCCGCTGG CGACGGGACATTATTA CTTTTGGTACGCGCTG TGACACTTCAAACTCG TACCGTGAGTAATAAT GCGCCGTCCACGGCAC CGCATCGAAAACGC	20	mir-126	TCGTACCGTGAGTAATAATGC	1076
mir-132	CGCGCCCCGCCCCCGC GTCTCCAGGGCAACCG TGGCTTTCGATTGTTA CTGTGGGAACTGGAGG TAACAGTCTACAGCCA TGGTCGCCCCGCAGCA CGCCCACGCGCCGC	21	miR-132 (RFAM-Human)	TAACAGTCTACAGCCATGGTCG	1077
mir-132	CGCGCCCCGCCCCCGC GTCTCCAGGGCAACCG TGGCTTTCGATTGTTA CTGTGGGAACTGGAGG TAACAGTCTACAGCCA TGGTCGCCCCGCAGCA CGCCCACGCGCCGC	21	mir-132	TAACAGTCTACAGCCATGGTCGC	206
mir-108_1	GCTGCCCGATGCACAC TGCAAGAACAATAAGG ATTTTTAGGGGCATTA TGACTGAGTCAGAAAA CACAGCTGCCCCTGAA AGTCCCTCATTTTTCT TGCTGTCCTTGAAC	22	mir-108	ATAAGGATTTTTAGGGGCATT	207
let-7i	ACACCATGGCCCTGGC TGAGGTAGTAGTTTGT GCTGTTGGTCGGGTTG TGACATTGCCCGCTGT GGAGATAACTGCGCAA GCTACTGCCTTGCTAG TGCTGGTGATGCTC	24	let-7i	TGAGGTAGTAGTTTGTGCT	209
let-7i	ACACCATGGCCCTGGC TGAGGTAGTAGTTTGT GCTGTTGGTCGGGTTG TGACATTGCCCGCTGT GGAGATAACTGCGCAA GCTACTGCCTTGCTAG TGCTGGTGATGCTC	24	let-7i_Ruvkun	TGAGGTAGTAGTTTGTGCTGTT	1078

mir-212	CGGGGCACCCCGCCCG GACAGCGCGCCGGCAC CTTGGCTCTAGACTGC TTACTGCCCGGGCCGC CCTCAGTAACAGTCTC CAGTCACGGCCACCGA CGCCTGGCCCCGCC	25	mir-212	TAACAGTCTCCAGTCACGGCC	210
hypothetical miRNA 023	AGATTTAATTAGCTCA GAGAAGAAATGTTGCT TGGGCAAGAGGACTTT TTAATTATCAGCTTGG ATAAATTTGAAAATGT TGATGCCTAGGGGTTG AGTTAATTAAAACC	26	hypothetical miRNA-023	TGGGCAAGAGGACTTTTTAAT	1079
mir-131_2/mir-9	GCCTGTGTGGGAAGCG AGTTGTTATCTTTGGT TATCTAGCTGTATGAG TGTATTGGTCTTCATA AAGCTAGATAACCGAA AGTAAAAACTCCTTCA AGATCGCCGGGGAG	27	mir-131	TAAAGCTAGATAACCGAAAGT	211
mir-131_2/mir-9	GCCTGTGTGGGAAGCG AGTTGTTATCTTTGGT TATCTAGCTGTATGAG TGTATTGGTCTTCATA AAGCTAGATAACCGAA AGTAAAAACTCCTTCA AGATCGCCGGGGAG	27	mir-131_Ruvkun	TAAAGCTAGATAACCGAAAGTA	1080
mir-131_2/mir-9	GCCTGTGTGGGAAGCG AGTTGTTATCTTTGGT TATCTAGCTGTATGAG TGTATTGGTCTTCATA AAGCTAGATAACCGAA AGTAAAAACTCCTTCA AGATCGCCGGGGAG	27	miR-9	TCTTTGGTTATCTAGCTGTATGA	1081
let-7b	GGCCGGGCCTGGCGGG GTGAGGTAGTAGGTTG TGTGGTTTCAGGGCAG TGATGTTGCCCCTCGG AAGATAACTATACAAC CTACTGCCTTCCCTGA GGAGCCCAGTGACA	28	let-7b	TGAGGTAGTAGGTTGTGTGGTT	212
let-7b	GGCCGGGCCTGGCGGG GTGAGGTAGTAGGTTG TGTGGTTTCAGGGCAG TGATGTTGCCCCTCGG AAGATAACTATACAAC CTACTGCCTTCCCTGA GGAGCCCAGTGACA	28	let-7b_Ruvkun	TGAGGTAGTAGGTTGTGTGGTTT	1082
mir-1d_1	CTGCATGCAGACTGCC TGCTTGGGAAACATAC TTCTTTATATGCCCAT ATGGACCTGCTAAGCT ATGGAATGTAAAGAAG TATGTATCTCAGGCCG GGACCTCTCTCGCC	29	miR-1 (RFAM)	TGGAATGTAAAGAAGTATGTA	1083
mir-1d_1	CTGCATGCAGACTGCC TGCTTGGGAAACATAC TTCTTTATATGCCCAT ATGGACCTGCTAAGCT ATGGAATGTAAAGAAG	29	mir-1d	TGGAATGTAAAGAAGTATGTAT	213

	TATGTATCTCAGGCCG GGACCTCTCTCGCC				
mir-122a	TGGCTACAGAGTTTCC TTAGCAGAGCTGTGGA GTGTGACAATGGTGTT TGTGTCTAAACTATCA AACGCCATTATCACAC TAAATAGCTACTGCTA GGCAATCCTTCCCT	30	miR-122a,b (Tuschl)	TGGAGTGTGACAATGGTGTTTG	1084
mir-122a	TGGCTACAGAGTTTCC TTAGCAGAGCTGTGGA GTGTGACAATGGTGTT TGTGTCTAAACTATCA AACGCCATTATCACAC TAAATAGCTACTGCTA GGCAATCCTTCCCT	30	mir-122a	TGGAGTGTGACAATGGTGTTTGT	214
mir-22	GCCCTCACCTGGCTGA GCCGCAGTAGTTCTTC AGTGGCAAGCTTTATG TCCTGACCCAGCTAAA GCTGCCAGTTGAAGAA CTGTTGCCCTCTGCCC CTGGCTTCGAGGAG	31	mir-22	AAGCTGCCAGTTGAAGAACTGT	215
hypothetical miRNA 30	CTACTGCTGTTGGTGG CAGCTTGGTGGTCGTA TGTGTGACGCCATTTA CTTGAACCTTTAGGAG TGACATCACATATACG GCAGCTAAACTGCTAC ATGGGACAACAATT	33	hypothetical miRNA-030	TGACATCACATATACGGCAGC	1085
mir-142	CGACGGACAGACAGAC AGTGCAGTCACCCATA AAGTAGAAAGCACTAC TAACAGCACTGGAGGG TGTAGTGTTTCCTACT TTATGGATGAGTGTAC TGTGGGCTTCGGAG	34	mir-142	CATAAAGTAGAAAGCACTAC	217
mir-142	CGACGGACAGACAGAC AGTGCAGTCACCCATA AAGTAGAAAGCACTAC TAACAGCACTGGAGGG TGTAGTGTTTCCTACT TTATGGATGAGTGTAC TGTGGGCTTCGGAG	34	miR-142-as	TGTAGTGTTTCCTACTTTATGG	1086
mir-142	CGACGGACAGACAGAC AGTGCAGTCACCCATA AAGTAGAAAGCACTAC TAACAGCACTGGAGGG TGTAGTGTTTCCTACT TTATGGATGAGTGTAC TGTGGGCTTCGGAG	34	miR-142as (Michael et al)	TGTAGTGTTTCCTACTTTATGGA	1087
mir-183	CCGCAGAGTGTGACTC CTGTTCTGTGTATGGC ACTGGTAGAATTCACT GTGAACAGTCTCAGTC AGTGAATTACCGAAGG GCCATAAACAGAGCAG AGACAGATCCACGA	35	mir-183	TATGGCACTGGTAGAATTCACTG	218
mir-214	GGCCTGGCTGGACAGA GTTGTCATGTGTCTGC CTGTCTACACTTGCTG	37	mir-214	ACAGCAGGCACAGACAGGCAG	219

	TGCAGAACATCCGCTC ACCTGTACAGCAGGCA CAGACAGGCAGTCACA TGACAACCCAGCCT				
mir-143	AGCAGCGCAGCGCCCT GTCTCCCAGCCTGAGG TGCAGTGCTGCATCTC TGGTCAGTTGGGAGTC TGAGATGAAGCACTGT AGCTCAGGAAGAGAGA AGTTGTTCTGCAGC	38	miR-143 (Michael et al)	TGAGATGAAGCACTGTAGCTC	1088
mir-143	AGCAGCGCAGCGCCCT GTCTCCCAGCCTGAGG TGCAGTGCTGCATCTC TGGTCAGTTGGGAGTC TGAGATGAAGCACTGT AGCTCAGGAAGAGAGA AGTTGTTCTGCAGC	38	mir-143	TGAGATGAAGCACTGTAGCTCA	220
mir-192_1	GCCGAGACCGAGTGCA CAGGGCTCTGACCTAT GAATTGACAGCCAGTG CTCTCGTCTCCCCTCT GGCTGCCAATTCCATA GGTCACAGGTATGTTC GCCTCAATGCCAGC	39	miR-192 (Tuschl)	CTGACCTATGAATTGACA	1089
mir-192_1	GCCGAGACCGAGTGCA CAGGGCTCTGACCTAT GAATTGACAGCCAGTG CTCTCGTCTCCCCTCT GGCTGCCAATTCCATA GGTCACAGGTATGTTC GCCTCAATGCCAGC	39	mir-192	CTGACCTATGAATTGACAGCC	221
mir-192_1	GCCGAGACCGAGTGCA CAGGGCTCTGACCTAT GAATTGACAGCCAGTG CTCTCGTCTCCCCTCT GGCTGCCAATTCCATA GGTCACAGGTATGTTC GCCTCAATGCCAGC	39	miR-192 (Michael et al)	TGACCTATGAATTGACAGCCAG	1090
hypothetical miRNA 039	CCCCTGTGCCTTGGGC GGGCGGCTGTTAAGAC TTGCAGTGATGTTTAA CTCCTCTCCACGTGAA CATCACAGCAAGTCTG TGCTGCTTCCCGTCCC TACGCTGCCTGGGC	42	hypothetical miRNA-039	TAAGACTTGCAGTGATGTTTA	1091
hypothetical miRNA 040	GCCAGCAAATAATGGC TGTTGTATTAGCTGCT TTTGATGATAGTATGA AAGAAGTATTAGCACT TGTCAACAAAACTGCT TACAACATAACATTAG CATGCATGGGCTGC	43	hypothetical miRNA-040	TGTCAACAAAACTGCTTACAA	1092
hypothetical miRNA 041	CATACACGGCTGTTAC ACAGGTTTTCCCATGA TAAGGCAATAGGTTAA TGAAATGCTCATTTCA TTTTACCAGTTGTTTT CTCTGTGAAGTTCCGA TAAGTAGCAAACCA	44	hypothetical miRNA-041	TACCAGTTGTTTTCTCTGTGA	1093

let-7a_3	CGACTGCCCTTTGGGG TGAGGTAGTAGGTTGT ATAGTTTGGGGCTCTG CCCTGCTATGGGATAA CTATACAATCTACTGT CTTTCCTGAAGTGGCT GTAATATCTGCGGT	45	let-7a	TGAGGTAGTAGGTTGTATAGTT	222
hypothetical miRNA 043	CCCCTTATAGGCCCAT TTTGACAGGAAATCTT TGAGAGGCAGCAGCAA TGAAGTGCCCAGAGAT TTCATCTGTCTTCTTT TGCTTTAGGAAATGCT GAGCGCAAGGCTCC	46	hypothetical miRNA-043	TGACAGGAAATCTTTGAGAGG	1094
hypothetical miRNA 044	GCCTGAAATGAAATTA CCATATTTTTAATCTT AATTTTCCACTCTGTT TATCTGACAGTGTGGA TGTGCAATCCAAACAG ATAATGAGAGAGTGGG ATATTGACACCGCT	47	hypothetical miRNA-044	TTCCACTCTGTTTATCTGACA	1095
mir-181a_1	AGAAGGGCTATCAGGC CAGCCTTCAGAGGACT CCAAGGAACATTCAAC GCTGTCGGTGAGTTTG GGATTTGAAAAAACCA CTGACCGTTGACTGTA CCTTGGGGTCCTTA	48	mir-178 (Kosik)	AACATTCAACGCTGTCGGTGAG	1096
mir-181a_1	AGAAGGGCTATCAGGC CAGCCTTCAGAGGACT CCAAGGAACATTCAAC GCTGTCGGTGAGTTTG GGATTTGAAAAAACCA CTGACCGTTGACTGTA CCTTGGGGTCCTTA	48	mir-181a	AACATTCAACGCTGTCGGTGAGT	223
let-7a_1	GTTCTCTTCACTGTGG GATGAGGTAGTAGGTT GTATAGTTTTAGGGTC ACACCCACCACTGGGA GATAACTATACAATCT ACTGTCTTTCCTAACG TGATAGAAAAGTCT	49	let-7a	TGAGGTAGTAGGTTGTATAGTT	222
mir-205	AAAGATCCTCAGACAA TCCATGTGCTTCTCTT GTCCTTCATTCCACCG GAGTCTGTCTCATACC CAACCAGATTTCAGTG GAGTGAAGTTCAGGAG GCATGGAGCTGACA	50	mir-205	TCCTTCATTCCACCGGAGTCTG	224
mir-33a	GGCCGCATACCTCCTG GCGGGCAGCTGTGGTG CATTGTAGTTGCATTG CATGTTCTGGTGGTAC CCATGCAATGTTTCCA CAGTGCATCACAGAGG CCTGCCTGGCCCTC	53	mir-33a	GTGCATTGTAGTTGCATTG	227
mir-196_2	TGCTCGCTCAGCTGAT CTGTGGCTTAGGTAGT TTCATGTTGTTGGGAT TGAGTTTTGAACTCGG CAACAAGAAACTGCCT	54	miR-196 (Tuschl)	TAGGTAGTTTCATGTTGTTGG	1097

	GAGTTACATCAGTCGG TTTTCGTCGAGGGC				
mir-196_2	TGCTCGCTCAGCTGAT CTGTGGCTTAGGTAGT TTCATGTTGTTGGGAT TGAGTTTTGAACTCGG CAACAAGAAACTGCCT GAGTTACATCAGTCGG TTTTCGTCGAGGGC	54	mir-196	TAGGTAGTTTCATGTTGTTGGG	228
let-7f_1	ATTGCTCTATCAGAGT GAGGTAGTAGATTGTA TAGTTGTGGGGTAGTG ATTTTACCCTGTTCAG GAGATAACTATACAAT CTATTGCCTTCCCTGA GGAGTAGACTTGCT	57	let-7f (Michael et al)	TGAGGTAGTAGATTGTATAGT	1098
let-7f_1	ATTGCTCTATCAGAGT GAGGTAGTAGATTGTA TAGTTGTGGGGTAGTG ATTTTACCCTGTTCAG GAGATAACTATACAAT CTATTGCCTTCCCTGA GGAGTAGACTTGCT	57	let-7f	TGAGGTAGTAGATTGTATAGTT	231
hypothetical miRNA 055	TTGAGCATGATGAATG ATTGGAGTCAGAGAAG CGGCGTGATAAATGGC AGCACCTTGGCTCCAT TGCATGCCCTATTGAT TCTCCTTCTTTATTAC TCCTACAACCCAGC	58	hypothetical miRNA-055	TTGCATGCCCTATTGATTCTC	1099
mir-29c	ACCACTGGCCCATCTC TTACACAGGCTGACCG ATTTCTCCTGGTGTTC AGAGTCTGTTTTTGTC TAGCACCATTTGAAAT CGGTTATGATGTAGGG GGAAAAGCAGCAGC	59	mir-29c	CTAGCACCATTTGAAATCGGTT	232
mir-29c	ACCACTGGCCCATCTC TTACACAGGCTGACCG ATTTCTCCTGGTGTTC AGAGTCTGTTTTTGTC TAGCACCATTTGAAAT CGGTTATGATGTAGGG GGAAAAGCAGCAGC	59	miR-29c (Tuschl)	TAGCACCATTTGAAATCGGTTA	1100
mir-130a	CCGGCATGCCTCTGCT GCTGGCCAGAGCTCTT TTCACATTGTGCTACT GTCTGCACCTGTCACT AGCAGTGCAATGTTAA AAGGGCATTGGCCGTG TAGTGCTACCCAGC	60	mir-130a	CAGTGCAATGTTAAAAGGGC	233
mir-130a	CCGGCATGCCTCTGCT GCTGGCCAGAGCTCTT TTCACATTGTGCTACT GTCTGCACCTGTCACT AGCAGTGCAATGTTAA AAGGGCATTGGCCGTG TAGTGCTACCCAGC	60	mir-130 (Kosik)	CAGTGCAATGTTAAAAGGGCAT	1101
hypothetical miRNA 058	TATCATCTTGTCAGAT GCTTAATGTTCTTCCT CCTGTCACTTTGGATA	61	hypothetical miRNA-	TGTCAGATGCTTAATGTTCTT	1102

	GGCCCAATTTGTAGAA TACTGCAGAGGTAAAA GAAGACAATTAACAGT GACAGGATGGTAAT		058		
mir-218_1	GTGATAATGTAGCGAG ATTTTCTGTTGTGCTT GATCTAACCATGTGGT TGCGAGGTATGAGTAA AACATGGTTCCGTCAA GCACCATGGAACGTCA CGCAGCTTTCTACA	62	mir-218	TTGTGCTTGATCTAACCATGT	234
mir-218_1	GTGATAATGTAGCGAG ATTTTCTGTTGTGCTT GATCTAACCATGTGGT TGCGAGGTATGAGTAA AACATGGTTCCGTCAA GCACCATGGAACGTCA CGCAGCTTTCTACA	62	mir-253* (Kosik)	TTGTGCTTGATCTAACCATGTG	1103
mir-124a_2	ATCAAGATTAGAGGCT CTGCTCTCCGTGTTCA CAGCGGACCTTGATTT AATGTCATACAATTAA GGCACGCGGTGAATGC CAAGAGCGGAGCCTAC GGCTGCACTTGAAG	63	mir-124a (Kosik)	TAAGGCACGCGGTGAATGCCA	1104
mir-124a_2	ATCAAGATTAGAGGCT CTGCTCTCCGTGTTCA CAGCGGACCTTGATTT AATGTCATACAATTAA GGCACGCGGTGAATGC CAAGAGCGGAGCCTAC GGCTGCACTTGAAG	63	mir-124a	TTAAGGCACGCGGTGAATGCCA	235
mir-124a_2	ATCAAGATTAGAGGCT CTGCTCTCCGTGTTCA CAGCGGACCTTGATTT AATGTCATACAATTAA GGCACGCGGTGAATGC CAAGAGCGGAGCCTAC GGCTGCACTTGAAG	63	mir-124a_Ruvkun	TTAAGGCACGCGGTGAATGCCAA	1105
mir-144	TCCTGTGCCCCCAGTG GGGCCCTGGCTGGGAT ATCATCATATACTGTA AGTTTGCGATGAGACA CTACAGTATAGATGAT GTACTAGTCCGGGCAC CCCCAGCTCTGGAG	66	mir-144	TACAGTATAGATGATGTACTAG	237
mir-221	TGAACATCCAGGTCTG GGGCATGAACCTGGCA TACAATGTAGATTTCT GTGTTCGTTAGGCAAC AGCTACATTGTCTGCT GGGTTTCAGGCTACCT GGAAACATGTTCTC	67	mir-221 (RFAM-mmu)	AGCTACATTGTCTGCTGGGTTT	1106
mir-221	TGAACATCCAGGTCTG GGGCATGAACCTGGCA TACAATGTAGATTTCT GTGTTCGTTAGGCAAC AGCTACATTGTCTGCT GGGTTTCAGGCTACCT GGAAACATGTTCTC	67	mir-221	AGCTACATTGTCTGCTGGGTTTC	238

mir-222	GCTGCTGGAAGGTGTA GGTACCCTCAATGGCT CAGTAGCCAGTGTAGA TCCTGTCTTTCGTAAT CAGCAGCTACATCTGG CTACTGGGTCTCTGAT GGCATCTTCTAGCT	68	mir-222 (RFAM-mmu)	AGCTACATCTGGCTACTGGGTCT	1107
mir-222	GCTGCTGGAAGGTGTA GGTACCCTCAATGGCT CAGTAGCCAGTGTAGA TCCTGTCTTTCGTAAT CAGCAGCTACATCTGG CTACTGGGTCTCTGAT GGCATCTTCTAGCT	68	mir-222	AGCTACATCTGGCTACTGGGTCTC	239
mir-30d	TCTTGTTCAGAAAGTC TGTTGTTGTAAACATC CCCGACTGGAAGCTGT AAGACACAGCTAAGCT TTCAGTCAGATGTTTG CTGCTACCGGCTATTC ACAGACATCCTCTT	69	mir-30d	TGTAAACATCCCCGACTGGAAG	240
mir-30d	TCTTGTTCAGAAAGTC TGTTGTTGTAAACATC CCCGACTGGAAGCTGT AAGACACAGCTAAGCT TTCAGTCAGATGTTTG CTGCTACCGGCTATTC ACAGACATCCTCTT	69	mir-30d_Ruvkun	TGTAAACATCCCCGACTGGAAGCT	1108
mir-128b	GCCCGGCAGCCACTGT GCAGTGGGAAGGGGGG CCGATACACTGTACGA GAGTGAGTAGCAGGTC TCACAGTGAACCGGTC TCTTTCCCTACTGTGT CACACTCCTAATGG	71	mir-128 (Kosik)	TCACAGTGAACCGGTCTCTTT	1073
mir-128b	GCCCGGCAGCCACTGT GCAGTGGGAAGGGGGG CCGATACACTGTACGA GAGTGAGTAGCAGGTC TCACAGTGAACCGGTC TCTTTCCCTACTGTGT CACACTCCTAATGG	71	mir-128b	TCACAGTGAACCGGTCTCTTTC	242
mir-219_2	GGGCCCTGAACTCAGG GGCTTCGCCACTGATT GTCCAAACGCAATTCT TGTACGAGTCTGCGGC CAACCGAGAATTGTGG CTGGACATCTGTGGCT GAGCTCCGGGCGCA	72	mir-219	TGATTGTCCAAACGCAATTCT	271
hypothetical miRNA 070	TTCGATGCTTGAAGAT GTCAGACTGTAGAATC TCTACGGGTAAGTGTG TGATTTCCTCAGTGAC ATCACATTTGCCTGCA GAGATTTTCCAGTCTG CCACTTTGAAGTTG	73	hypothetical miRNA-070	TCACATTTGCCTGCAGAGATT	1109
mir-129_2	GGCATATTCTGCCCTT CGCGAATCTTTTTGCG GTCTGGGCTTGCTGTA CATAACTCAATAGCCG GAAGCCCTTACCCCAA	76	mir-129as/mir-258* (Kosik)	AAGCCCTTACCCCAAAAAGCAT	1110

	AAAGCATTTGCGGAGG GCGCACTCGTCGAG				
mir-129_2	GGCATATTCTGCCCTT CGCGAATCTTTTTGCG GTCTGGGCTTGCTGTA CATAACTCAATAGCCG GAAGCCCTTACCCCAA AAAGCATTTGCGGAGG GCGCACTCGTCGAG	76	mir-129	CTTTTTGCGGTCTGGGCTTGC	243
mir-129_2	GGCATATTCTGCCCTT CGCGAATCTTTTTGCG GTCTGGGCTTGCTGTA CATAACTCAATAGCCG GAAGCCCTTACCCCAA AAAGCATTTGCGGAGG GCGCACTCGTCGAG	76	miR-129b (RFAM-Human)	CTTTTTGCGGTCTGGGCTTGCT	1111
mir-133b	CAGAAGAAAGATGCCC CCTGCTCTGGCTGGTC AAACGGAACCAAGTCC GTCTTCCTGAGAGGTT TGGTCCCCTTCAACCA GCTACAGCAGGGCTGG CAATGCCCAGTCCT	77	mir-133b	TTGGTCCCCTTCAACCAGCTA	244
hypothetical miRNA 075	AGCGCAGCTTTAATTA CTCATGCTGCTGGTTA AAATATTAATGGGGCA CAGAGTGTTGCATGCT CATTTCTGTTGATTTT TAATTAGCAGTAATTC ATTTTGCACAAAGC	78	hypothetical miRNA-075	TGGTTAAAATATTAATGGGGC	1112
let-7d	AAAAAAATGGGTTCCT AGGAAGAGGTAGTAGG TTGCATAGTTTTAGGG CAGGGATTTTGCCCAC AAGGAGGTAACTATAC GACCTGCTGCCTTTCT TAGGGCCTTATTAT	79	let-7d	AGAGGTAGTAGGTTGCATAGT	245
let-7d	AAAAAAATGGGTTCCT AGGAAGAGGTAGTAGG TTGCATAGTTTTAGGG CAGGGATTTTGCCCAC AAGGAGGTAACTATAC GACCTGCTGCCTTTCT TAGGGCCTTATTAT	79	let-7d_Ruvkun	AGAGGTAGTAGGTTGCATAGTT	1113
let-7d	AAAAAAATGGGTTCCT AGGAAGAGGTAGTAGG TTGCATAGTTTTAGGG CAGGGATTTTGCCCAC AAGGAGGTAACTATAC GACCTGCTGCCTTTCT TAGGGCCTTATTAT	79	let-7d* (RFAM-M. mu.)	CTATACGACCTGCTGCCTTTCT	1114
mir-15b	AATCCTACATTTTTGA GGCCTTAAAGTACTGT AGCAGCACATCATGGT TTACATGCTACAGTCA AGATGCGAATCATTAT TTGCTGCTCTAGAAAT TTAAGGAAATTCAT	80	miR-15b (Michael et al)	TAGCAGCACATCATGGTTTAC	1115
mir-15b	AATCCTACATTTTTGA GGCCTTAAAGTACTGT AGCAGCACATCATGGT	80	mir-15b	TAGCAGCACATCATGGTTTACA	246

	TTACATGCTACAGTCA AGATGCGAATCATTAT TTGCTGCTCTAGAAAT TTAAGGAAATTCAT				
mir-29a	ACGACCTTCTGTGACC CCTTAGAGGATGACTG ATTTCTTTTGGTGTTC AGAGTCAATATAATTT TCTAGCACCATCTGAA ATCGGTTATAATGATT GGGGAAGAGCACCA	81	mir-29a	CTAGCACCATCTGAAATCGGTT	247
mir-29a	ACGACCTTCTGTGACC CCTTAGAGGATGACTG ATTTCTTTTGGTGTTC AGAGTCAATATAATTT TCTAGCACCATCTGAA ATCGGTTATAATGATT GGGGAAGAGCACCA	81	mir-29a_Ruvkun	TAGCACCATCTGAAATCGGTTA	1116
hypothetical miRNA 079	CAAAGCTCTCCTGCCT GCTTCTGTGTGATATG TTTGATATTGGGTTGT TTAATTAGGAACCAAC TAAATGTCAAACATAT TCTTACAGCAGCAGGT GATTCAGCACCACC	82	hypothetical miRNA-079	TGATATGTTTGATATTGGG	1117
mir-199b	CCAGAGGACACCTCCA CTCCGTCTACCCAGTG TTTAGACTATCTGTTC AGGACTCCCAAATTGT ACAGTAGTCTGCACAT TGGTTAGGCTGGGCTG GGTTAGACCCTCGG	83	mir-199b (human)	CCCAGTGTTTAGACTATCTGTTC	248
mir-199b	CCAGAGGACACCTCCA CTCCGTCTACCCAGTG TTTAGACTATCTGTTC AGGACTCCCAAATTGT ACAGTAGTCTGCACAT TGGTTAGGCTGGGCTG GGTTAGACCCTCGG	83	miR-199-as	TACAGTAGTCTGCACATTGGTT	1118
mir-129_1	GGATGGCTGCTGTCTC CTTTGGATCTTTTTGC GGTCTGGGCTTGCTGT TCCTCTCAACAGTAGT CAGGAAGCCCTTACCC CAAAAAGTATCTGCGG GAGGCCTTGTCCAC	84	mir-129	CTTTTTGCGGTCTGGGCTTGC	243
mir-129_1	GGATGGCTGCTGTCTC CTTTGGATCTTTTTGC GGTCTGGGCTTGCTGT TCCTCTCAACAGTAGT CAGGAAGCCCTTACCC CAAAAAGTATCTGCGG GAGGCCTTGTCCAC	84	miR-129b (RFAM-Human)	CTTTTTGCGGTCTGGGCTTGCT	1111
let-7e	ACCTGCCGCGCCCCCC GGGCTGAGGTAGGAGG TTGTATAGTTGAGGAG GACACCCAAGGAGATC ACTATACGGCCTCCTA GCTTTCCCCAGGCTGC GCCCTGCACGGGAC	85	let-7e	TGAGGTAGGAGGTTGTATAGT	249

hypothetical miRNA 083	TGGCAGGTTGTTTAGT TTTTTCGTTTGAAGGT TTTCATTAGTCTAATG AGGACTGTGCAAGGGC GAGCAGTCAGCACAAT TTACATGGGGAAGCTA TCATAATAAATGAA	86	hypothetical miRNA-083	TTACATGGGGAAGCTATCATA	1119
let-7c_1	AGCTGTGTGCATCCGG GTTGAGGTAGTAGGTT GTATGGTTTAGAGTTA CACCCTGGGAGTTAAC TGTACAACCTTCTAGC TTTCCTTGGAGCACAC TTGAGCCGTCGAGG	87	let-7c	TGAGGTAGTAGGTTGTATGGTT	250
let-7c_1	AGCTGTGTGCATCCGG GTTGAGGTAGTAGGTT GTATGGTTTAGAGTTA CACCCTGGGAGTTAAC TGTACAACCTTCTAGC TTTCCTTGGAGCACAC TTGAGCCGTCGAGG	87	let-7c_Ruvkun	TGAGGTAGTAGGTTGTATGGTTT	1120
mir-204	GGCTACAGTCTTTCTT CATGTGACTCGTGGAC TTCCCTTTGTCATCCT ATGCCTGAGAATATAT GAAGGAGGCTGGGAAG GCAAAGGGACGTTCAA TTGTCATCACTGGC	88	mir-204	TTCCCTTTGTCATCCTATGCCT	251
mir-204	GGCTACAGTCTTTCTT CATGTGACTCGTGGAC TTCCCTTTGTCATCCT ATGCCTGAGAATATAT GAAGGAGGCTGGGAAG GCAAAGGGACGTTCAA TTGTCATCACTGGC	88	miR-204 (Tuschl)	TTCCCTTTGTCATCCTATGCCTG	1121
mir-145	CCACTCGCTCCCACCT TGTCCTCACGGTCCAG TTTTCCCAGGAATCCC TTAGATGCTAAGATGG GGATTCCTGGAAATAC TGTTCTTGAGGTCATG GTTTCACAGCTGGA	89	miR-145 (Michael et al)	GTCCAGTTTTCCCAGGAATCC	1122
mir-145	CCACTCGCTCCCACCT TGTCCTCACGGTCCAG TTTTCCCAGGAATCCC TTAGATGCTAAGATGG GGATTCCTGGAAATAC TGTTCTTGAGGTCATG GTTTCACAGCTGGA	89	mir-145	GTCCAGTTTTCCCAGGAATCCCTT	252
mir-124a_1	TCCTTCCTCAGGAGAA AGGCCTCTCTCTCCGT GTTCACAGCGGACCTT GATTTAAATGTCCATA CAATTAAGGCACGCGG TGAATGCCAAGAATGG GGCTGGCTGAGCAC	90	mir-124a (Kosik)	TAAGGCACGCGGTGAATGCCA	1104
mir-124a_1	TCCTTCCTCAGGAGAA AGGCCTCTCTCTCCGT GTTCACAGCGGACCTT GATTTAAATGTCCATA CAATTAAGGCACGCGG	90	mir-124a	TTAAGGCACGCGGTGAATGCCA	235

	TGAATGCCAAGAATGG GGCTGGCTGAGCAC				
mir-124a_1	TCCTTCCTCAGGAGAA AGGCCTCTCTCTCCGT GTTCACAGCGGACCTT GATTTAAATGTCCATA CAATTAAGGCACGCGG TGAATGCCAAGAATGG GGCTGGCTGAGCAC	90	mir-124a_Ruvkun	TTAAGGCACGCGGTGAATGCCAA	1105
DiGeorge syndrome critical region gene 8/ hypothetical miRNA-088	AGTCGCCAGTCACTTA AGCTGAGTGCATTGTG ATTTCCAATAATTGAG GCAGTGGTTCTAAAAG CTGTCTACATTAATGA AAAGAGCAATGTGGCC AGCTTGACTAAGCC	91	hypothetical miRNA-088	TGTGATTTCCAATAATTGAGG	1123
mir-213/ mir-181a_2	TGAGTTTTGAGGTTGC TTCAGTGAACATTCAA CGCTGTCGGTGAGTTT GGAATTAAAATCAAAA CCATCGACCGTTGATT GTACCCTATGGCTAAC CATCATCTACTCCA	92	mir-178 (Kosik)	AACATTCAACGCTGTCGGTGAG	1096
mir-213/ mir-181a_2	TGAGTTTTGAGGTTGC TTCAGTGAACATTCAA CGCTGTCGGTGAGTTT GGAATTAAAATCAAAA CCATCGACCGTTGATT GTACCCTATGGCTAAC CATCATCTACTCCA	92	mir-181a	AACATTCAACGCTGTCGGTGAGT	223
mir-213/ mir-181a_2	TGAGTTTTGAGGTTGC TTCAGTGAACATTCAA CGCTGTCGGTGAGTTT GGAATTAAAATCAAAA CCATCGACCGTTGATT GTACCCTATGGCTAAC CATCATCTACTCCA	92	mir-213	ACCATCGACCGTTGATTGTACC	253
hypothetical miRNA 090	CAGCGATACATTAATG CTCATTTGGCTCTGCA AATCTTACCGTTTGCT TAGGCCAAATGGCGCA TCAATGACTATCGCTC TTACAAAGCTCTTGAA TCAGTATTATGTAA	93	hypothetical miRNA-090	TAGGCCAAATGGCGCATCAAT	1124
mir-20	TATCTGATGTGACAGC TTCTGTAGCACTAAAG TGCTTATAGTGCAGGT AGTGTTTAGTTATCTA CTGCATTATGAGCACT TAAAGTACTGCTAGCT GTAGAACTCCAGCT	94	miR-20* (human)	ACTGCATTATGAGCACTTAAA	1125
mir-20	TATCTGATGTGACAGC TTCTGTAGCACTAAAG TGCTTATAGTGCAGGT AGTGTTTAGTTATCTA CTGCATTATGAGCACT TAAAGTACTGCTAGCT GTAGAACTCCAGCT	94	miR-20 (RFAM-Human)	TAAAGTGCTTATAGTGCAGGTA	1126
mir-20	TATCTGATGTGACAGC TTCTGTAGCACTAAAG	94	mir-20	TAAAGTGCTTATAGTGCAGGTAG	254

	TGCTTATAGTGCAGGT AGTGTTTAGTTATCTA CTGCATTATGAGCACT TAAAGTACTGCTAGCT GTAGAACTCCAGCT				
mir-133a_1	CTAGCAGCACTACAAT GCTTTGCTAGAGCTGG TAAAATGGAACCAAAT CGCCTCTTCAATGGAT TTGGTCCCCTTCAACC AGCTGTAGCTATGCAT TGATTACTACGGGA	95	mir-133a	TTGGTCCCCTTCAACCAGCTGT	255
mir-138_2	GCGGAGTTCTGGTATC GTTGCTGCAGCTGGTG TTGTGAATCAGGCCGA CGAGCAGCGCATCCTC TTACCCGGCTATTTCA CGACACCAGGGTTGCA TCATACCCATCCTC	96	mir-138	AGCTGGTGTTGTGAATC	256
mir-138_2	GCGGAGTTCTGGTATC GTTGCTGCAGCTGGTG TTGTGAATCAGGCCGA CGAGCAGCGCATCCTC TTACCCGGCTATTTCA CGACACCAGGGTTGCA TCATACCCATCCTC	96	mir-138_Ruvkun	AGCTGGTGTTGTGAATCAGGCCG	1127
mir-196_1	CTAGAGCTTGAATTGG AACTGCTGAGTGAATT AGGTAGTTTCATGTTG TTGGGCCTGGGTTTCT GAACACAACAACATTA AACCACCCGATTCACG GCAGTTACTGCTCC	98	miR-196 (Tuschl)	TAGGTAGTTTCATGTTGTTGG	1097
mir-196_1	CTAGAGCTTGAATTGG AACTGCTGAGTGAATT AGGTAGTTTCATGTTG TTGGGCCTGGGTTTCT GAACACAACAACATTA AACCACCCGATTCACG GCAGTTACTGCTCC	98	mir-196	TAGGTAGTTTCATGTTGTTGGG	228
mir-125b_1	AACATTGTTGCGCTCC TCTCAGTCCCTGAGAC CCTAACTTGTGATGTT TACCGTTTAAATCCAC GGGTTAGGCTCTTGGG AGCTGCGAGTCGTGCT TTTGCATCCTGGAA	99	mir-125b	TCCCTGAGACCCTAACTTGTGA	258
mir-199a_2	AGGAAGCTTCTGGAGA TCCTGCTCCGTCGCCC CAGTGTTCAGACTACC TGTTCAGGACAATGCC GTTGTACAGTAGTCTG CACATTGGTTAGACTG GGCAAGGGAGAGCA	100	miR-199-s	CCCAGTGTTCAGACTACCTGTT	1128
mir-199a_2	AGGAAGCTTCTGGAGA TCCTGCTCCGTCGCCC CAGTGTTCAGACTACC TGTTCAGGACAATGCC GTTGTACAGTAGTCTG CACATTGGTTAGACTG GGCAAGGGAGAGCA	100	mir-199a	CCCAGTGTTCAGACTACCTGTTC	259

mir-199a_2	AGGAAGCTTCTGGAGA TCCTGCTCCGTCGCCC CAGTGTTCAGACTACC TGTTCAGGACAATGCC GTTGTACAGTAGTCTG CACATTGGTTAGACTG GGCAAGGGAGAGCA	100	miR-199-as	TACAGTAGTCTGCACATTGGTT	1118
hypothetical miRNA 099	CTGGGGAGGTGAGCCT GAAAACAAAGGCAGAT AGAGAAGTCACAGCTC ACTGGTGAGGGAGCTA GAGAGTTGTTTTCTTA ATACCCTCTGCCTTTG AATCTGCCTAGATT	102	hypothetical miRNA-099	AGGCAGATAGAGAAGTCACAG	1272
mir-181b_1	CCTGTGCAGAGATTAT TTTTTAAAAGGTCACA ATCAACATTCATTGCT GTCGGTGGGTTGAACT GTGTGGACAAGCTCAC TGAACAATGAATGCAA CTGTGGCCCCGCTT	103	mir-181b	AACATTCATTGCTGTCGGTGGGTT	260
hypothetical miRNA 101	GTATATTCAGGGACAG GCCATTGACAGTCAAT TAACAAGTTTGATTGG TATGTCAACTCATTCT TTTGAATTGTTAATAG TATGTTAATAGCATTC GTTTCTTTGTGCAG	104	hypothetical miRNA-101	TGACAGTCAATTAACAAGTTT	1130
mir-141	CTGTCGGCCGGCCCTG GGTCCATCTTCCAGTA CAGTGTTGGATGGTCT AATTGTGAAGCTCCTA ACACTGTCTGGTAAAG ATGGCTCCCGGGTGGG TTCTCTCGGCAGTA	105	mir-141	AACACTGTCTGGTAAAGATGG	261
mir-131_1/mir-9	GCCAGGAGGCGGGGTT GGTTGTTATCTTTGGT TATCTAGCTGTATGAG TGGTGTGGAGTCTTCA TAAAGCTAGATAACCG AAAGTAAAAATAACCC CATACACTGCGCAG	106	mir-131	TAAAGCTAGATAACCGAAAGT	211
mir-131_1/mir-9	GCCAGGAGGCGGGGTT GGTTGTTATCTTTGGT TATCTAGCTGTATGAG TGGTGTGGAGTCTTCA TAAAGCTAGATAACCG AAAGTAAAAATAACCC CATACACTGCGCAG	106	mir-131_Ruvkun	TAAAGCTAGATAACCGAAAGTA	1080
mir-131_1/mir-9	GCCAGGAGGCGGGGTT GGTTGTTATCTTTGGT TATCTAGCTGTATGAG TGGTGTGGAGTCTTCA TAAAGCTAGATAACCG AAAGTAAAAATAACCC CATACACTGCGCAG	106	miR-9	TCTTTGGTTATCTAGCTGTATGA	1081
mir-133a_2	TCGGATCTGGGAGCCA AATGCTTTGCTAGAGC TGGTAAAATGGAACCA AATCGACTGTCCAATG GATTTGGTCCCCTTCA	107	mir-133a	TTGGTCCCCTTCAACCAGCTGT	255

	ACCAGCTGTAGCTGTG CATTGATGGCGCCG				
hypothetical miRNA 105	CCGCCTCAGAGCCGCC CGCCGTTCCTTTTTCC TATGCATATACTTCTT TGAGGATCTGGCCTAA AGAGGTATAGGGCATG GGAAAACGGGGCGGTC GGGTCCTCCCCAGC	108	miR-202 (human)	AGAGGTATAGGGCATGGGAAAA	1131
hypothetical miRNA 105	CCGCCTCAGAGCCGCC CGCCGTTCCTTTTTCC TATGCATATACTTCTT TGAGGATCTGGCCTAA AGAGGTATAGGGCATG GGAAAACGGGGCGGTC GGGTCCTCCCCAGC	108	hypothetical miRNA-105	TTCCTATGCATATACTTCTTT	1132
hypothetical miRNA 107	CTATAATGCTTAGATT ATCAATCATCTTGACA GTTTATTGGCTTTATC ACCACACATACCATTA AAATGATGTCTGGCCT AGACTGTCAGGAGCAA ACATTAAACAGACC	110	hypothetical miRNA-107	TGACAGTTTATTGGCTTTATC	1133
mir-1d_2	ACAGCTAACAACTTAG TAATACCTACTCAGAG TACATACTTCTTTATG TACCCATATGAACATA CAATGCTATGGAATGT AAAGAAGTATGTATTT TTGGTAGGCAATAA	111	miR-1 (RFAM)	TGGAATGTAAAGAAGTATGTA	1083
mir-1d_2	ACAGCTAACAACTTAG TAATACCTACTCAGAG TACATACTTCTTTATG TACCCATATGAACATA CAATGCTATGGAATGT AAAGAAGTATGTATTT TTGGTAGGCAATAA	111	mir-1d	TGGAATGTAAAGAAGTATGTAT	213
mir-1d_2	ACAGCTAACAACTTAG TAATACCTACTCAGAG TACATACTTCTTTATG TACCCATATGAACATA CAATGCTATGGAATGT AAAGAAGTATGTATTT TTGGTAGGCAATAA	111	miR-1d (Tuschl)	TGGAATGTAAAGAAGTATGTATT	1134
mir-220	GACAGTGTGGCATTGT AGGGCTCCACACCGTA TCTGACACTTTGGGCG AGGGCACCATGCTGAA GGTGTTCATGATGCGG TCTGGGAACTCCTCAC GGATCTTACTGATG	113	mir-220	CCACACCGTATCTGACACTTT	263
hypothetical miRNA 111	CTCTGGCCTCCGCTTC CTCCTCCTCCGACTCG GACACCGGCGGAGCCT CCCCGCCCCCGCGGAA GAAACCCCGAGCCTCG GCGGCGGAGGGAGTAG GAGAGCCCGGGGCT	114	hypothetical miRNA-111	TTCCTCCTCCTCCGACTCGGA	1135
mir-7_3	AGATTAGAGTGGCTGT GGTCTAGTGCTGTGTG GAAGACTAGTGATTTT	115	mir-7	TGGAAGACTAGTGATTTTGTT	198

	GTTGTTCTGATGTACT ACGACAACAAGTCACA GCCGGCCTCATAGCGC AGACTCCCTTCGAC				
mir-218_2	GACCAGTCGCTGCGGG GCTTTCCTTTGTGCTT GATCTAACCATGTGGT GGAACGATGGAAACGG AACATGGTTCTGTCAA GCACCGCGGAAAGCAC CGTGCTCTCCTGCA	116	mir-218	TTGTGCTTGATCTAACCATGT	234
mir-218_2	GACCAGTCGCTGCGGG GCTTTCCTTTGTGCTT GATCTAACCATGTGGT GGAACGATGGAAACGG AACATGGTTCTGTCAA GCACCGCGGAAAGCAC CGTGCTCTCCTGCA	116	mir-253* (Kosik)	TTGTGCTTGATCTAACCATGTG	1103
mir-211	TCACCTGGCCATGTGA CTTGTGGGCTTCCCTT TGTCATCCTTCGCCTA GGGCTCTGAGCAGGGC AGGGACAGCAAAGGGG TGCTCAGTTGTCACTT CCCACAGCACGGAG	120	mir-211 (human)	TTCCCTTTGTCATCCTTCGCCT	1136
mir-30b	CCAAGTTTCAGTTCAT GTAAACATCCTACACT CAGCTGTAATACATGG ATTGGCTGGGAGGTGG ATGTTTACTTCAGCTG ACTTGGAATGTCAACC AATTAACATTGATA	122	mir-30b	TGTAAACATCCTACACTCAGC	266
mir-30b	CCAAGTTTCAGTTCAT GTAAACATCCTACACT CAGCTGTAATACATGG ATTGGCTGGGAGGTGG ATGTTTACTTCAGCTG ACTTGGAATGTCAACC AATTAACATTGATA	122	mir-30b_Ruvkun	TGTAAACATCCTACACTCAGCT	1137
hypothetical miRNA 120	GGCTTCTTCCAGTCAT CCTGAGGTAGATATCA TCCAGGAATGCTGAGG CCTTATGGCTTACAGC AATCCAGTAATGATAT AAAAGGTGATTGGAGG TTAGATTTACATTG	123	hypothetical miRNA-120	TTACAGCAATCCAGTAATGAT	1138
mir-10a	GATCTGTCTGTCTTCT GTATATACCCTGTAGA TCCGAATTTGTGTAAG GAATTTTGTGGTCACA AATTCGTATCTAGGGG AATATGTAGTTGACAT AAACACTCCGCTCT	125	mir-10a (Tuschl)	TACCCTGTAGATCCGAATTTGT	1139
mir-10a	GATCTGTCTGTCTTCT GTATATACCCTGTAGA TCCGAATTTGTGTAAG GAATTTTGTGGTCACA AATTCGTATCTAGGGG AATATGTAGTTGACAT AAACACTCCGCTCT	125	mir-10a	TACCCTGTAGATCCGAATTTGTG	267

let-7f_2	ACACTGGTGCTCTGTG GGATGAGGTAGTAGAT TGTATAGTTTTAGGGT CATACCCCATCTTGGA GATAACTATACAGTCT ACTGTCTTTCCCACGG TGGTACACTTCTTC	127	let-7f (Michael et al)	TGAGGTAGTAGATTGTATAGT	1098
let-7f_2	ACACTGGTGCTCTGTG GGATGAGGTAGTAGAT TGTATAGTTTTAGGGT CATACCCCATCTTGGA GATAACTATACAGTCT ACTGTCTTTCCCACGG TGGTACACTTCTTC	127	let-7f	TGAGGTAGTAGATTGTATAGTT	231
mir-108_2	CCGAGGAATACTGCAA GAGCAATAAGGATTTT TAGGGGCATTATGATA GTGGAATGGAAACACA TCTGCCCCCAAAAGTC CCTCATTTTCCCTGCG GTAACGAACCAGCT	129	mir-108	ATAAGGATTTTTAGGGGCATT	207
mir-137	CTTGGTCCTCTGACTC TCTTCGGTGACGGGTA TTCTTGGGTGGATAAT ACGGATTACGTTGTTA TTGCTTAAGAATACGC GTAGTCGAGGAGAGTA CCAGCGGCAGGGGG	130	mir-137	TATTGCTTAAGAATACGCGTAG	270
mir-148b	CATTTCCAAGCACGAT TAGCATTTGAGGTGAA GTTCTGTTATACACTC AGGCTGTGGCTCTCTG AAAGTCAGTGCATCAC AGAACTTTGTCTCGAA AGCTTTCTAGCAGC	132	mir-148b	TCAGTGCATCACAGAACTTTGT	272
mir-130b	GGGGAGGCACTGGCAG GCCTGCCCGACACTCT TTCCCTGTTGCACTAC TATAGGCCGCTGGGAA GCAGTGCAATGATGAA AGGGCATCGGTCAGGT CCAGCCTGCTACCC	133	mir-130b	CAGTGCAATGATGAAAGGGC	273
mir-130b	GGGGAGGCACTGGCAG GCCTGCCCGACACTCT TTCCCTGTTGCACTAC TATAGGCCGCTGGGAA GCAGTGCAATGATGAA AGGGCATCGGTCAGGT CCAGCCTGCTACCC	133	mir-266* (Kosik)	CAGTGCAATGATGAAAGGGCAT	1140
let-7a_4	TTGTGACTGCATGCTC CCAGGTTGAGGTAGTA GGTTGTATAGTTTAGA ATTACATCAAGGGAGA TAACTGTACAGCCTCC TAGCTTTCCTTGGGTC TTGCACTAAACAAC	135	let-7a	TGAGGTAGTAGGTTGTATAGTT	222
mir-216	GATGGCTGTGAGTTGG CTTAATCTCAGCTGGC AACTGTGAGATGTTCA TACAATCCCTCACAGT GGTCTCTGGGATTATG	136	mir-216	TAATCTCAGCTGGCAACTGTG	274

	CTAAACAGAGCAATTT CCTAGCCCTCACGA				
hypothetical miRNA 137	GTTCAACATAAGCAAA CAGATTGTAAACTGGC TGATAATTTTTGTACT GACAATGTCATTTACA GCTGTCAGCCTTTCGT CTGCCTTGTTTGCTTT ATTCAAATATGAAC	140	hypothetical miRNA-137	TAAACTGGCTGATAATTTTTG	1141
hypothetical miRNA 138	CCCTCCAATGTCTGAT TAATCAAGCCTGCAAA CAGCTTATTTCTTTTA GCCTGCATGCAAGTAT GAAAATGAGATTCTGG GAGCCGAACATGGTGC AGATTTGTTCATTC	141	hypothetical miRNA-138	TGCAAGTATGAAAATGAGATT	1142
mir-124a_3	CCCGCCCCAGCCCTGA GGGCCCCTCTGCGTGT TCACAGCGGACCTTGA TTTAATGTCTATACAA TTAAGGCACGCGGTGA ATGCCAAGAGAGGCGC CTCCGCCGCTCCTT	143	mir-124a (Kosik)	TAAGGCACGCGGTGAATGCCA	1104
mir-124a_3	CCCGCCCCAGCCCTGA GGGCCCCTCTGCGTGT TCACAGCGGACCTTGA TTTAATGTCTATACAA TTAAGGCACGCGGTGA ATGCCAAGAGAGGCGC CTCCGCCGCTCCTT	143	mir-124a	TTAAGGCACGCGGTGAATGCCA	235
mir-124a_3	CCCGCCCCAGCCCTGA GGGCCCCTCTGCGTGT TCACAGCGGACCTTGA TTTAATGTCTATACAA TTAAGGCACGCGGTGA ATGCCAAGAGAGGCGC CTCCGCCGCTCCTT	143	mir-124a_Ruvkun	TTAAGGCACGCGGTGAATGCCAA	1105
mir-7_2	CTGGATACAGAGTGGA CCGGCTGGCCCCATCT GGAAGACTAGTGATTT TGTTGTTGTCTTACTG CGCTCAACAACAAATC CCAGTCTACCTAATGG TGCCAGCCATCGCA	144	mir-7	TGGAAGACTAGTGATTTTGTT	198
hypothetical miRNA 142	GGGGTGAATTTATTTC TTACAGAACCGCCCTG ATTCAGATGGTGCAAG CCTCGCAGGCCAGAAA CATCTTCCTGACGCTG CTCCCCACCTTCTGCC CTCTCTTTCCCAGC	145	hypothetical miRNA-142	TGACGCTGCTCCCCACCTTCT	1143
hypothetical miRNA 143	GCTGATGAAAATAGGG CAGTGGTTTAAATAGA TTTGCAAGCAATTTAC CTTTTCACAATGTTGG CAATCTGATGCAATTT GCTTGCAATTTTGTCT GCTTTCAGTAGCAC	146	hypothetical miRNA-143	TGCAATTTGCTTGCAATTTTG	1144
mir-210	ACCCGGCAGTGCCTCC AGGCGCAGGGCAGCCC CTGCCCACCGCACACT	148	mir-210	CTGTGCGTGTGACAGCGGCTG	277

	GCGCTGCCCCAGACCC ACTGTGCGTGTGACAG CGGCTGATCTGTGCCT GGGCAGCGCGACCC				
mir-215	ATCATTCAGAAATGGT ATACAGGAAAATGACC TATGAATTGACAGACA ATATAGCTGAGTTTGT CTGTCATTTCTTTAGG CCAATATTCTGTATGA CTGTGCTACTTCAA	149	mir-215	ATGACCTATGAATTGACAGAC	278
mir-223	CCTGGCCTCCTGCAGT GCCACGCTCCGTGTAT TTGACAAGCTGAGTTG GACACTCCATGTGGTA GAGTGTCAGTTTGTCA AATACCCCAAGTGCGG CACATGCTTACCAG	150	mir-223	TGTCAGTTTGTCAAATACCCC	279
mir-131_3/mir-9	CACGGCGCGGCAGCGG CACTGGCTAAGGGAGG CCCGTTTCTCTCTTTG GTTATCTAGCTGTATG AGTGCCACAGAGCCGT CATAAAGCTAGATAAC CGAAAGTAGAAATG	151	mir-131	TAAAGCTAGATAACCGAAAGT	211
mir-131_3/mir-9	CACGGCGCGGCAGCGG CACTGGCTAAGGGAGG CCCGTTTCTCTCTTTG GTTATCTAGCTGTATG AGTGCCACAGAGCCGT CATAAAGCTAGATAAC CGAAAGTAGAAATG	151	mir-131_Ruvkun	TAAAGCTAGATAACCGAAAGTA	1080
mir-131_3/mir-9	CACGGCGCGGCAGCGG CACTGGCTAAGGGAGG CCCGTTTCTCTCTTTG GTTATCTAGCTGTATG AGTGCCACAGAGCCGT CATAAAGCTAGATAAC CGAAAGTAGAAATG	151	miR-9	TCTTTGGTTATCTAGCTGTATGA	1081
mir-199a_1	TGGATAGCCGGCCCCG CCAACCCAGTGTTCAG ACTACCTGTTCAGGAG GCTCTCAATGTGTACA GTAGTCTGCACATTGG TTAGGCTGGGCTTGGG TGAGCGGCTCGTCG	152	miR-199-s	CCCAGTGTTCAGACTACCTGTT	1128
mir-199a_1	TGGATAGCCGGCCCCG CCAACCCAGTGTTCAG ACTACCTGTTCAGGAG GCTCTCAATGTGTACA GTAGTCTGCACATTGG TTAGGCTGGGCTTGGG TGAGCGGCTCGTCG	152	mir-199a	CCCAGTGTTCAGACTACCTGTTC	259
mir-199a_1	TGGATAGCCGGCCCCG CCAACCCAGTGTTCAG ACTACCTGTTCAGGAG GCTCTCAATGTGTACA GTAGTCTGCACATTGG TTAGGCTGGGCTTGGG TGAGCGGCTCGTCG	152	miR-199-as	TACAGTAGTCTGCACATTGGTT	1118

mir-30c_1	CCTAGAGAGCACTGAG CGACAGATACTGTAAA CATCCTACACTCTCAG CTGTGGAAAGTAAGAA AGCTGGGAGAAGGCTG TTTACTCTTTCTGCCT TGGAAGTCAACTAA	153	mir-30c	TGTAAACATCCTACACTCTCAGC	280
mir-30c_1	CCTAGAGAGCACTGAG CGACAGATACTGTAAA CATCCTACACTCTCAG CTGTGGAAAGTAAGAA AGCTGGGAGAAGGCTG TTTACTCTTTCTGCCT TGGAAGTCAACTAA	153	mir-30c_Ruvkun	TGTAAACATCCTACACTCTCAGCT	1129
hypothetical miRNA 153	TGTTGAATGCAAGCAG ATGCTGATAATATCAG AAGTCACAGCATAATT TTTTTTGATCAAAGGG CTCAAGTGAGCCTGAT GAAGCATGCATCTTGC TCGTCTTTGATAAA	156	hypothetical miRNA-153	TGCAAGCAGATGCTGATAATA	1145
hypothetical miRNA 154	CCTGCAGTGATGCTTC ATGAGCAAATCACATG ATGTCAGAATGGTATG GTTTCGATTTAATCAA GAAAGAGATTAAAGTG GATGTGTGTTATTTTC AACTTCGCAGCAGC	157	hypothetical miRNA-154	TTAAAGTGGATGTGTGTTATT	1146
mir-26b	CGCCCCACCCTGCCCG GGACCCAGTTCAAGTA ATTCAGGATAGGTTGT GTGCTGTCCAGCCTGT TCTCCATTACTTGGCT CGGGGACCGGTGCCCT GCAGCCTTGGGGTG	158	miR-26b (RFAM-Human)	TTCAAGTAATTCAGGATAGGT	1147
mir-26b	CGCCCCACCCTGCCCG GGACCCAGTTCAAGTA ATTCAGGATAGGTTGT GTGCTGTCCAGCCTGT TCTCCATTACTTGGCT CGGGGACCGGTGCCCT GCAGCCTTGGGGTG	158	mir-26b	TTCAAGTAATTCAGGATAGGTT	281
hypothetical miRNA 156	TGCGTTTACATAACAC CAGGCGTGTGGGAGCT GGAGGAAGAGGTTGCG AATGTAGGAGAGATAA GGCTCCTGCTTTCCCT CCTTCCTTCTTGGTGG TACCAGGCTTGACA	159	hypothetical miRNA-156	TGCTTTCCCTCCTTCCTTCTT	1148
mir-152	GGCCCGCTGTCCCCCC CGGCCCAGGTTCTGTG ATACACTCCGACTCGG GCTCTGGAGCAGTCAG TGCATGACAGAACTTG GGCCCGGAAGGACCTT CTGCACCCAACGGG	160	mir-152	TCAGTGCATGACAGAACTTGG	282
mir-135_1	CAGCCCCAGGCCTCGC TGTTCTCTATGGCTTT TTATTCCTATGTGATT CTACTGCTCACTCATA TAGGGATTGGAGCCGT	161	miR-135 (RFAM-Human)	TATGGCTTTTTATTCCTATGTGA	1149

	GGCGCACGGCGGGGAC AGCCAGCGGAGGGT				
mir-135_1	CAGCCCCAGGCCTCGC TGTTCTCTATGGCTTT TTATTCCTATGTGATT CTACTGCTCACTCATA TAGGGATTGGAGCCGT GGCGCACGGCGGGGAC AGCCAGCGGAGGGT	161	mir-135	TATGGCTTTTTATTCCTATGTGAT	283
non-coding RNA in rhabdomyosarcoma/ mir-135_2	ACCAAGATAAATTCAC TCTAGTGCTTTATGGC TTTTTATTCCTATGTG ATAGTAATAAAGTCTC ATGTAGGGATGGAAGC CATGAAATACATTGTG AAAAATCATCAACT	162	miR-135 (RFAM-Human)	TATGGCTTTTTATTCCTATGTGA	1149
non-coding RNA in rhabdomyosarcoma/ mir-135_2	ACCAAGATAAATTCAC TCTAGTGCTTTATGGC TTTTTATTCCTATGTG ATAGTAATAAAGTCTC ATGTAGGGATGGAAGC CATGAAATACATTGTG AAAAATCATCAACT	162	mir-135	TATGGCTTTTTATTCCTATGTGAT	283
mir-217	AGTATAATTATTACAT AGTTTTTGATGTCGCA GATACTGCATCAGGAA CTGATTGGATAAGAAT CAGTCACCATCAGTTC CTAATGCATTGCCTTC AGCATCTAAACAAG	163	mir-217 (human)	TACTGCATCAGGAACTGATTGGAT	284
hypothetical miRNA 161	CTTGGCCATAAACTTG TAGTCATCCTCTATCC AATCATATTGTCTTGA GTAATTAAAATGATTA GCTTAATTAGCTTAAT TAACTAAATTTGACTA CAGGACATGGCCAT	164	hypothetical miRNA-161	TGGCCATAAACTTGTAGTCAT	1150
mir-15a	GGCGCGAATGTGTGTT TAAAAAAAATAAAACC TTGGAGTAAAGTAGCA GCACATAATGGTTTGT GGATTTTGAAAAGGTG CAGGCCATATTGTGCT GCCTCAAAAATACA	165	mir-15_Ruvkun	TAGCAGCACATAATGGTTTGT	1151
mir-15a	GGCGCGAATGTGTGTT TAAAAAAAATAAAACC TTGGAGTAAAGTAGCA GCACATAATGGTTTGT GGATTTTGAAAAGGTG CAGGCCATATTGTGCT GCCTCAAAAATACA	165	mir-15a	TAGCAGCACATAATGGTTTGTG	269
let-7g	TTTGCCTGATTCCAGG CTGAGGTAGTAGTTTG TACAGTTTGAGGGTCT ATGATACCACCCGGTA CAGGAGATAACTGTAC AGGCCACTGCCTTGCC AGGAACAGCGCGCC	166	let-7g	TGAGGTAGTAGTTTGTACAGT	285
let-7g	TTTGCCTGATTCCAGG CTGAGGTAGTAGTTTG TACAGTTTGAGGGTCT	166	let-7gL_Ruvkun	TGAGGTAGTAGTTTGTACAGTT	1152

	ATGATACCACCCGGTA CAGGAGATAACTGTAC AGGCCACTGCCTTGCC AGGAACAGCGCGCC				
hypothetical miRNA 164	AATTGTCCTTGGTTTT ACAATGATAAATGAAA AACATTAAAATTCTCC AACTGAACAGGTATGC AAGGATTTTTATGTTT TGTTGTTGTTGTTAAA ACAGTGAGAGCAAA	167	hypothetical miRNA-164	TGCAAGGATTTTTATGTTTTG	1153
hypothetical miRNA 166	GCAGTGGCGCTCAATG CTGTGCACTTCCAGTT GCAGCACCTGTAAGGT TTGTTAAAGGTAAAGG CAGGTCGGGAAAAGGT GCTTCGAGGAAGAGGC CTGGGAGGGGGCGA	169	hypothetical miRNA-166	TTCCAGTTGCAGCACCTGTAA	1154
hypothetical miRNA 168_1/similar to ribosomal protein L5	ATGGACAAGATCTATG ACGGCCAAGTGGAGGT GACTGGCGATAAATAC AATGTGGAAAGTACTG ATGGTCAGCCAGGTGC CTTCACCTGCTGTATG GATGCAGGTCTTGC	171	hypothetical miRNA-168	AGCCAGGTGCCTTCACCTGCT	1155
forkhead box P2/hypothetical miRNA-169	CACTGGAGGCTGTTCT ATAAATGATCATTGAA GGGCTGCAAGCTAGCC TATAATTACAGGAAAG AAAGTGGCAGCTCTGG CATTTCATAACTATGT GTCCTCGAAAAGTG	172	hypothetical miRNA-169	TGGCAGCTCTGGCATTTCATA	1156
hypothetical miRNA 170	GAATGTATGATCTTGC TCTAACACTTGGCCAG ACCTGTGTCACCCACT GCTAGTGCCTGAAGTC GACAGACAATTCTGCC AAGGTAACCGAGAATC ATTAAGCATCCTGC	173	hypothetical miRNA-170	TGATCTTGCTCTAACACTTGG	1157
glutamate receptor, ionotropic, AMPA 2 / hypothetical miRNA-171	CACCCTGTCTGACAAG TATGTTTTATCGTTTC AAGAAATGCGGTTAAC CTCGCAGTACTAAAAC TGAATGAACAAGGCCT GTTGGACAAATTGAAA AACAAATGGTGGTA	174	hypothetical miRNA-171	TGACAAGTATGTTTTATCGTT	1158
hypothetical miRNA 172	TGTTTTTTTGAGTACA TGTGTATAAATAGAGG TGGCTTCCTGTCAGTT TGGTATTATTGATATG ATCCAACTGCAAGAAG TTACTGCAACACTTTG CATCTTAAAGGTCC	175	hypothetical miRNA-172	TCCAACTGCAAGAAGTTACT	1159
hypothetical miRNA 173	TAGTTCAGCACTCTTA CCTCTTATTGGTGTAC CACCTGGGTGGATAAT ATGAATGCAAATAAGA TTAGAAAGAAGAAGCA TTAGTACGAGAAGAAG GAGGCTAGGGCTGG	176	hypothetical miRNA-173	TAGTACGAGAAGAAGGAGGCT	1160

mir-182	GAGCTGCTTGCCTCCC CCCGTTTTTGGCAATG GTAGAACTCACACTGG TGAGGTAACAGGATCC GGTGGTTCTAGACTTG CCAACTATGGGGCGAG GACTCAGCCGGCAC	177	miR-182* (RFAM-Human)	TGGTTCTAGACTTGCCAACTA	1161
mir-182	GAGCTGCTTGCCTCCC CCCGTTTTTGGCAATG GTAGAACTCACACTGG TGAGGTAACAGGATCC GGTGGTTCTAGACTTG CCAACTATGGGGCGAG GACTCAGCCGGCAC	177	mir-182	TTTGGCAATGGTAGAACTCACA	287
hypothetical miRNA 175	CTTGCCAGAAACATCA GTGACATGGACAAAGG TGTCATTGAAGGAGAC AAAGATGTGGCAGGCA CCAAATACATTCTCTC CTTCAACCACCTGAGG TCCGAGGCTGATGA	178	hypothetical miRNA-175	TCTCCTTCAACCACCTGAGGT	1162
hypothetical miRNA 176	TGGAAGGAAAATAGGA GTTTGATATGACATAT TGTGTGTCTCAGCAAG ACTCATAAATAATTTT GACAAGTTTTTGTATG CATGGGAAAGTCCTTG ATTCAGCCTCCCAT	179	hypothetical miRNA-176	TAGGAGTTTGATATGACATAT	1163
hypothetical miRNA-177_1	GGGAACCAGCGCTTTC AGTAAGAGAGTGGTAC CACGTGTCTTCAAAAT GAAACGTTTCTTGGAG ACAAACATGCTACTCT CACTGAGTACACAAGC TTCCTGGTTGTCAG	180	hypothetical miRNA-177	AGACAAACATGCTACTCTCAC	1164
hypothetical miRNA 178	CCAGTTTCCATCTGTC ATGATAGCCTATCTCC GAACCTTCAATCTGTC AAAAGCTCGCTGCCTG GCTGAAGGCTCCAGGA GATTTGGTGCACTAAA CACATTTGACAACA	181	hypothetical miRNA-178	TAGCCTATCTCCGAACCTTCA	1165
hypothetical miRNA 179	AATGCCAGTGAGTTTG AAAGGCACTTTGTCCA ATTAGAAGTGTGGAGA AATATTCATCCTGTCC ATGACAAAGATGAAGT GCTTCTTTCAAAAGCG GCGGTGGCAGGCTG	182	hypothetical miRNA-179	TGAAAGGCACTTTGTCCAATT	1166
hypothetical miRNA 181	TTGTGCACCTCACCTG CTCTGGAAGTAGTTTG CTAGCTCTGATGCTTC ATGGTTCAGACTCCTC AGGTGCACGATTAAAT TTCCAGAGTTGGTGAA CATGGCGCCACATG	184	hypothetical miRNA-181	TCACCTGCTCTGGAAGTAGTT	1167
mir-148a	GGAGGAAGACAGCACG TTTGGTCTTTTGAGGC AAAGTTCTGAGACACT CCGACTCTGAGTATGA TAGAAGTCAGTGCACT	185	mir-148a	TCAGTGCACTACAGAACTTTGT	288

	ACAGAACTTTGTCTCT AGAGGCTGTGGTCG				
hypothetical miRNA 185	ACACAAAACATGAACT GTGTACTCATTGTCTT CGCTGCACAGCTTGGC ATTGGGGTTGGTGACT CTGATGGCCAGCTGAG CAGCTCTTTCCACAAT GGCTTTGTGGTCCT	188	hypothetical miRNA-185	TGATGGCCAGCTGAGCAGCTC	1168
hypothetical miRNA-177_2/ hypothetical miRNA 186	ATATGGGAACCAGTGC TTGCAGAAAGAGGGTA GTTCCACATGTCTGCA AAACGAGACATCTCTT GAAGACAAACATGCTA CTCTCACTGCGTACAT AAGCTTCCTATTTG	189	hypothetical miRNA-177	AGACAAACATGCTACTCTCAC	1164
mir-181c	CGGAAAATTTGCCAAG GGTTTGGGGGAACATT CAACCTGTCGGTGAGT TTGGGCAGCTCAGGCA AACCATCGACCGTTGA GTGGACCCTGAGGCCT GGAATTGCCATCCT	190	mir-181c	AACATTCAACCTGTCGGTGAGT	290
hypothetical miRNA 188	AGAATGGTATCATAGG ACAGTGTGATGGAATT TTTCTTTCTCTGTCAT CATTAAGGGGGTTCCC CCTATGGTGAGGGGAA TGAAAAGTACGATTTA ATGTTCTCTGGAGA	191	hypothetical miRNA-188	TGGTGAGGGGAATGAAAAGTA	1169
mir-100_1	CCTGTTGCCACAAACC CGTAGATCCGAACTTG TGGTATTAGTCCGCAC AAGCTTGTATCTATAG GTATGTGTCTGTTAGG	945	mir-100	AACCCGTAGATCCGAACTTGTG	275
mir-101_1	TGCCCTGGCTCAGTTA TCACAGTGCTGATGCT GTCTATTCTAAAGGTA CAGTACTGTGATAACT GAAGGATGGCA	946	mir-101	TACAGTACTGTGATAACTGA	265
mir-101_1	TGCCCTGGCTCAGTTA TCACAGTGCTGATGCT GTCTATTCTAAAGGTA CAGTACTGTGATAACT GAAGGATGGCA	946	miR-101 (RFAM-Human)	TACAGTACTGTGATAACTGAAG	1170
mir-101_3	TGTCCTTTTTCGGTTA TCATGGTACCGATGCT GTATATCTGAAAGGTA CAGTACTGTGATAACT GAAGAATGGTG	947	mir-101	TACAGTACTGTGATAACTGA	265
mir-101_3	TGTCCTTTTTCGGTTA TCATGGTACCGATGCT GTATATCTGAAAGGTA CAGTACTGTGATAACT GAAGAATGGTG	947	miR-101 (RFAM-Human)	TACAGTACTGTGATAACTGAAG	1170
mir-29b_2	CTTCAGGAAGCTGGTT TCATATGGTGGTTTAG ATTTAAATAGTGATTG TCTAGCACCATTTGAA ATCAGTGTTCTTGGGG G	948	miR-29b (RFAM-Human)	TAGCACCATTTGAAATCAGT	1172

mir-29b_2	CTTCAGGAAGCTGGTT TCATATGGTGGTTTAG ATTTAAATAGTGATTG TCTAGCACCATTTGAA ATCAGTGTTCTTGGGG G	948	miR-29b (RFAM-M. mu.)	TAGCACCATTTGAAATCAGTGT	1173
mir-29b_2	CTTCAGGAAGCTGGTT TCATATGGTGGTTTAG ATTTAAATAGTGATTG TCTAGCACCATTTGAA ATCAGTGTTCTTGGGG G	948	mir-29b	TAGCACCATTTGAAATCAGTGTT	195
mir-29b_1	CTTCTGGAAGCTGGTT TCACATGGTGGCTTAG ATTTTTCCATCTTTGT ATCTAGCACCATTTGA AATCAGTGTTTTAGGA G	949	miR-29b (RFAM-Human)	TAGCACCATTTGAAATCAGT	1172
mir-29b_1	CTTCTGGAAGCTGGTT TCACATGGTGGCTTAG ATTTTTCCATCTTTGT ATCTAGCACCATTTGA AATCAGTGTTTTAGGA G	949	miR-29b (RFAM-M. mu.)	TAGCACCATTTGAAATCAGTGT	1173
mir-29b_1	CTTCTGGAAGCTGGTT TCACATGGTGGCTTAG ATTTTTCCATCTTTGT ATCTAGCACCATTTGA AATCAGTGTTTTAGGA G	949	mir-29b	TAGCACCATTTGAAATCAGTGTT	195
mir-103_1	TACTGCCCTCGGCTTC TTTACAGTGCTGCCTT GTTGCATATGGATCAA GCAGCATTGTACAGGG CTATGAAGGCATTG	950	mir-103	AGCAGCATTGTACAGGGCTATGA	225
mir-106	CCTTGGCCATGTAAAA GTGCTTACAGTGCAGG TAGCTTTTTGAGATCT ACTGCAATGTAAGCAC TTCTTACATTACCATG G	951	mir-106 (human)	AAAAGTGCTTACAGTGCAGGTAGC	230
mir-107	CTCTCTGCTTTCAGCT TCTTTACAGTGTTGCC TTGTGGCATGGAGTTC AAGCAGCATTGTACAG GGCTATCAAAGCACAG A	952	mir-107	AGCAGCATTGTACAGGGCTATCA	229
mir-16_1	GCAGTGCCTTAGCAGC ACGTAAATATTGGCGT TAAGATTCTAAAATTA TCTCCAGTATTAACTG TGCTGCTGAAGTAAGG T	953	mir-16	TAGCAGCACGTAAATATTGGCG	196
mir-16_1	GCAGTGCCTTAGCAGC ACGTAAATATTGGCGT TAAGATTCTAAAATTA TCTCCAGTATTAACTG TGCTGCTGAAGTAAGG T	953	mir-16_Ruvkun	TAGCAGCACGTAAATATTGGCGT	1176
mir-16_3	GTTCCACTCTAGCAGC ACGTAAATATTGGCGT	954	mir-16	TAGCAGCACGTAAATATTGGCG	196

	AGTGAAATATATATTA AACACCAATATTACTG TGCTGCTTTAGTGTGA C				
mir-16_3	GTTCCACTCTAGCAGC ACGTAAATATTGGCGT AGTGAAATATATATTA AACACCAATATTACTG TGCTGCTTTAGTGTGA C	954	mir-16_Ruvkun	TAGCAGCACGTAAATATTGGCGT	1176
mir-18	TTTTTGTTCTAAGGTG CATCTAGTGCAGATAG TGAAGTAGATTAGCAT CTACTGCCCTAAGTGC TCCTTCTGGCATAAGA A	955	mir-18	TAAGGTGCATCTAGTGCAGATA	262
mir-18	TTTTTGTTCTAAGGTG CATCTAGTGCAGATAG TGAAGTAGATTAGCAT CTACTGCCCTAAGTGC TCCTTCTGGCATAAGA A	955	mir-18_Ruvkun	TAAGGTGCATCTAGTGCAGATAG	1177
mir-19a	CAGTCCTCTGTTAGTT TTGCATAGTTGCACTA CAAGAAGAATGTAGTT GTGCAAATCTATGCAA AACTGATGGTGGCCTG	956	mir-19a	TGTGCAAATCTATGCAAAACTGA	268
mir-19b_1	TTCTATGGTTAGTTTT GCAGGTTTGCATCCAG CTGTGTGATATTCTGC TGTGCAAATCCATGCA AAACTGACTGTGGTAG	957	mir-19b* (Michael et al)	AGTTTTGCAGGTTTGCATCCAGC	1179
mir-19b_1	TTCTATGGTTAGTTTT GCAGGTTTGCATCCAG CTGTGTGATATTCTGC TGTGCAAATCCATGCA AAACTGACTGTGGTAG	957	mir-19b	TGTGCAAATCCATGCAAAACTGA	241
mir-19b_2	TTACAATTAGTTTTGC AGGTTTGCATTTCAGC GTATATATGTATATGT GGCTGTGCAAATCCAT GCAAAACTGATTGTGA T	958	mir-19b	TGTGCAAATCCATGCAAAACTGA	241
mir-21	ACCTTGTCGGGTAGCT TATCAGACTGATGTTG ACTGTTGAATCTCATG GCAACACCAGTCGATG GGCTGTCTGACATTTT G	959	mir-21	TAGCTTATCAGACTGATGTTGA	236
mir-23a	CCACGGCCGGCTGGGG TTCCTGGGGATGGGAT TTGCTTCCTGTCACAA ATCACATTGCCAGGGA TTTCCAACCGACCCTG A	960	mir-23a	ATCACATTGCCAGGGATTTCC	289
mir-24_2	CCCTGGGCTCTGCCTC CCGTGCCTACTGAGCT GAAACACAGTTGGTTT GTGTACACTGGCTCAG TTCAGCAGGAACAGGG G	961	mir-24	TGGCTCAGTTCAGCAGGAACAG	264

mir-17/mir-91	TCAGAATAATGTCAAAGTGCTTACAGTGCAGGTAGTGATATGTGCATCTACTGCAGTGAAGGCACTTGTAGCATTATGGTGA	962	mir-17 (human, rat)	ACTGCAGTGAAGGCACTTGT	1180
mir-17/mir-91	TCAGAATAATGTCAAAGTGCTTACAGTGCAGGTAGTGATATGTGCATCTACTGCAGTGAAGGCACTTGTAGCATTATGGTGA	962	mir-91_Ruvkun	CAAAGTGCTTACAGTGCAGGTAG	1181
mir-17/mir-91	TCAGAATAATGTCAAAGTGCTTACAGTGCAGGTAGTGATATGTGCATCTACTGCAGTGAAGGCACTTGTAGCATTATGGTGA	962	mir-17as/mir-91	CAAAGTGCTTACAGTGCAGGTAGT	204
mir-92_1	CTTTCTACACAGGTTGGGATCGGTTGCAATGCTGTGTTTCTGTATGGTATTGCACTTGTCCCGGCCTGTTGAGTTTGG	963	miR-92 (RFAM-M. mu.)	TATTGCACTTGTCCCGGCCTG	1182
mir-92_1	CTTTCTACACAGGTTGGGATCGGTTGCAATGCTGTGTTTCTGTATGGTATTGCACTTGTCCCGGCCTGTTGAGTTTGG	963	mir-92	TATTGCACTTGTCCCGGCCTGT	216
mir-96	TGGCCGATTTTGGCACTAGCACATTTTTGCTTGTGTCTCTCCGCTCTGAGCAATCATGTGCAGTGCCAATATGGGAAA	964	mir-96	TTTGGCACTAGCACATTTTTGC	203
mir-96	TGGCCGATTTTGGCACTAGCACATTTTTGCTTGTGTCTCTCCGCTCTGAGCAATCATGTGCAGTGCCAATATGGGAAA	964	miR-96 (RFAM-M. mu.)	TTTGGCACTAGCACATTTTTGCT	1183
mir-30a	GTGAGCGACTGTAAACATCCTCGACTGGAAGCTGTGAAGCCACAGATGGGCTTTCAGTCGGATGTTTGCAGCTGCCTACT	965	mir-30a	CTTTCAGTCGGATGTTTGCAGC	193
mir-30a	GTGAGCGACTGTAAACATCCTCGACTGGAAGCTGTGAAGCCACAGATGGGCTTTCAGTCGGATGTTTGCAGCTGCCTACT	965	miR-30a-s	TGTAAACATCCTCGACTGGAAGC	1184
mir-98	GTGAGGTAGTAAGTTGTATTGTTGTGGGGTAGGGATATTAGGCCCCAATTAGAAGATAACTATACAACTTACTACTTTCC	966	mir-98	TGAGGTAGTAAGTTGTATTGTT	257
mir-104 (Mourelatos)	AAATGTCAGACAGCCCATCGACTGGTGTTGCCATGAGATTCAACAGTCAACATCAGTCTGATAAGCTACCCGACAAGG	967	miR-104 (Mourelatos)	TCAACATCAGTCTGATAAGCTA	335
mir-105 (Mourelatos)	TGTGCATCGTGGTCAAATGCTCAGACTCCTGTGGTGGCTGCTCATGCA	968	miR-105 (Mourelatos)	TCAAATGCTCAGACTCCTGT	1185

	CCACGGATGTTTGAGC ATGTGCTACGGTGTCT A				
mir-27 (Mourelatos)	CCTGAGGAGCAGGGCT TAGCTGCTTGTGAGCA GGGTCCACACCAAGTC GTGTTCACAGTGGCTA AGTTCCGCCCCCCAGG	969	miR-27 (Mourelatos)	TTCACAGTGGCTAAGTTCC	1186
mir-27 (Mourelatos)	CCTGAGGAGCAGGGCT TAGCTGCTTGTGAGCA GGGTCCACACCAAGTC GTGTTCACAGTGGCTA AGTTCCGCCCCCCAGG	969	miR-27a (RFAM-M. mu.)	TTCACAGTGGCTAAGTTCCGC	1187
mir-27 (Mourelatos)	CCTGAGGAGCAGGGCT TAGCTGCTTGTGAGCA GGGTCCACACCAAGTC GTGTTCACAGTGGCTA AGTTCCGCCCCCCAGG	969	miR-27a (RFAM-Human)	TTCACAGTGGCTAAGTTCCGCC	1188
mir-92_2	TCATCCCTGGGTGGGG ATTTGTTGCATTACTT GTGTTCTATATAAAGT ATTGCACTTGTCCCGG CCTGTGGAAGA	970	miR-92 (RFAM-M. mu.)	TATTGCACTTGTCCCGGCCTG	1182
mir-92_2	TCATCCCTGGGTGGGG ATTTGTTGCATTACTT GTGTTCTATATAAAGT ATTGCACTTGTCCCGG CCTGTGGAAGA	970	mir-92	TATTGCACTTGTCCCGGCCTGT	216
mir-93 (Mourelatos)	CTGGGGGCTCCAAAGT GCTGTTCGTGCAGGTA GTGTGATTACCCAACC TACTGCTGAGCTAGCA CTTCCCGAGCCCCCGG	971	miR-93 (Mourelatos)	AAAGTGCTGTTCGTGCAGGTAG	1189
mir-93 (Mourelatos)	CTGGGGGCTCCAAAGT GCTGTTCGTGCAGGTA GTGTGATTACCCAACC TACTGCTGAGCTAGCA CTTCCCGAGCCCCCGG	971	miR-93 (Tuschl)	CAAAGTGCTGTTCGTGC	1190
mir-93 (Mourelatos)	CTGGGGGCTCCAAAGT GCTGTTCGTGCAGGTA GTGTGATTACCCAACC TACTGCTGAGCTAGCA CTTCCCGAGCCCCCGG	971	miR-93 (RFAM-M. mu.)	CAAAGTGCTGTTCGTGCAGGTAG	1191
mir-95 (Mourelatos)	AACACAGTGGGCACTC AATAAATGTCTGTTGA ATTGAAATGCGTTACA TTCAACGGGTATTTAT TGAGCACCCACTCTGT G	972	miR-95 (Mourelatos)	TTCAACGGGTATTTATTGAGCA	1192
mir-99 (Mourelatos)	CCCATTGGCATAAACC CGTAGATCCGATCTTG TGGTGAAGTGGACCGC ACAAGCTCGCTTCTAT GGGTCTGTGTCAGTGT G	973	miR-99 (Mourelatos)	AACCCGTAGATCCGATCTTGTG	1193
mir-99 (Mourelatos)	CCCATTGGCATAAACC CGTAGATCCGATCTTG TGGTGAAGTGGACCGC ACAAGCTCGCTTCTAT GGGTCTGTGTCAGTGT G	973	miR-99a (Tuschl)	ACCCGTAGATCCGATCTTGT	1194

mir-25	CTCCCTCACAGGACAG CTGAACTCCGGGACTG GCCAGTGTTGAGAGGC GGAGACTTGGGCAATT GCTGGACGCTGCCCTG GGCATTGCACTTGTCT CGGTCTGACAGTGCCG GCCCAACACTGCGGAT GCTGGGGGGGAGGGGG	974	miR-25 (Tuschl)	CATTGCACTTGTCTCGGTCTGA	1195
mir-28	TTTAAAATGGTCCTTG CCCTCAAGGAGCTCAC AGTCTATTGAGTTACC TTTCTGACTTTCCCAC TAGATTGTGAGCTCCT GGAGGGCAGGCACTTT CGTTC	975	miR-28 (Tuschl)	AAGGAGCTCACAGTCTATTGAG	1196
mir-31	CTCCTGTAACTTGGAA CTGGAGAGGAGGCAAG ATGCTGGCATAGCTGT TGAACTGGGAACCTGC TATGCCAACATATTGC CATCTTTCCTGTCTGA CAGCAGCCAT	976	miR-31 (RFAM-M. mu.)	AGGCAAGATGCTGGCATAGCTG	1197
mir-31	CTCCTGTAACTTGGAA CTGGAGAGGAGGCAAG ATGCTGGCATAGCTGT TGAACTGGGAACCTGC TATGCCAACATATTGC CATCTTTCCTGTCTGA CAGCAGCCAT	976	miR-31 (Tuschl)	GGCAAGATGCTGGCATAGCTG	1198
mir-32	TTCTGCTTGCTCTGGT GGAGATATTGCACATT ACTAAGTTGCATGTTG TCACGGCCTCAATGCA ATTTAGTGTGTGTGAT ATTTTCACATGAGTGC ATGCA	977	miR-32 (Tuschl)	TATTGCACATTACTAAGTTGC	1199
mir-149	GGCCGGCGCCCGAGCT CTGGCTCCGTGTCTTC ACTCCCGTGCTTGTCC GAGGAGGGAGGGAGGG ACGGGGGCTGTGCTGG GGCAGCTGGAACA	978	miR-149	TCTGGCTCCGTGTCTTCACTCC	1200
mir-30c_2	AACCATGCTGTAGTGT GTGTAAACATCCTACA CTCTCAGCTGTGAGCT CAAGGTGGCTGGGAGA GGGTTGTTTACTCCTT CTGCCATGGAAAACAT CAGCT	979	mir-30c	TGTAAACATCCTACACTCTCAGC	280
mir-30c_2	AACCATGCTGTAGTGT GTGTAAACATCCTACA CTCTCAGCTGTGAGCT CAAGGTGGCTGGGAGA GGGTTGTTTACTCCTT CTGCCATGGAAAACAT CAGCT	979	mir-30c_Ruvkun	TGTAAACATCCTACACTCTCAGCT	1129
mir-99b	TCCTGGGTCCTGGCAC CCACCCGTAGAACCGA CCTTGCGGGGCCTTCG CCGCACACAAGCTCGT	980	miR-99b	CACCCGTAGAACCGACCTTGCG	1201

	GTCTGTGGGTCCGTGT CGGGGGCTCACCATCG				
MiR-125a	TCTAGGTCCCTGAGAC CCTTTAACCTGTGAGG ACATCCAGGGTCACAG GTGAGGTTCTTGGGAG CCTGGCGT	981	miR-125a	TCCCTGAGACCCTTTAACCTGTG	1202
MiR-125b_2	GACTTTTCCTAGTCCC TGAGACCCTAACTTGT GAGGTATTTTAGTAAC ATCACAAGTCAGGCTC TTGGGACCTAGGCGGA G	982	mir-125b	TCCCTGAGACCCTAACTTGTGA	258
mir-26a_2	CCCATAGAGGCTGTGG CTGGATTCAAGTAATC CAGGATAGGCTGTTTC CATCTGTGAGGCCTAT TCTTGATTACTTGTTT CTGGAGGCAGCTGATG GTC	983	miR-26a (Michael et al)	TTCAAGTAATCCAGGATAGGC	1203
mir-26a_2	CCCATAGAGGCTGTGG CTGGATTCAAGTAATC CAGGATAGGCTGTTTC CATCTGTGAGGCCTAT TCTTGATTACTTGTTT CTGGAGGCAGCTGATG GTC	983	mir-26a	TTCAAGTAATCCAGGATAGGCT	226
mir-127	TCTCCAGCCTGCTGAA GCTCAGAGGGCTCTGA TTCAGAAAGATCATCG GATCCGTCTGAGCTTG GCTGGTCGGAAGT	984	mir-127_Ruvkun	TCGGATCCGTCTGAGCTTGG	1204
mir-127	TCTCCAGCCTGCTGAA GCTCAGAGGGCTCTGA TTCAGAAAGATCATCG GATCCGTCTGAGCTTG GCTGGTCGGAAGT	984	miR-127	TCGGATCCGTCTGAGCTTGGCT	1205
mir-136	TGGATGAGCCCTCGGA GGACTCCATTTGTTTT GATGATGGATTCTTAT GCTCCATCATCGTCTC AAATGAGTCTTCAGAG GGTTCTATCAT	985	miR-136	ACTCCATTTGTTTTGATGATGGA	1206
mir-154	GTGGTACTTGAAGATA GGTTATCCGTGTTGCC TTCGCTTTATTTGTGA CGAATCATACACGGTT GACCTATTTTTCAGTA CCAA	986	miR-154	TAGGTTATCCGTGTTGCCTTCG	1207
mir-26a_1	CCGTGGCCTCGTTCAA GTAATCCAGGATAGGC TGTGCAGGTCCCAATG GGCCTATTCTTGGTTA CTTGCACGGGGACGCG G	987	miR-26a (Michael et al)	TTCAAGTAATCCAGGATAGGC	1203
mir-26a_1	CCGTGGCCTCGTTCAA GTAATCCAGGATAGGC TGTGCAGGTCCCAATG GGCCTATTCTTGGTTA CTTGCACGGGGACGCG G	987	mir-26a	TTCAAGTAATCCAGGATAGGCT	226

mir_186	ATTGCTTGTAACTTTC CAAAGAATTCTCCTTT TGGGCTTTCTGGTTTT ATTTTAAGCCCAAAGG TGAATTTTTTGGGAAG TTTGAGCT	988	miR-186	CAAAGAATTCTCCTTTTGGGCTT	1208
mir_198	GGTTCTGATCATTGGT CCAGAGGGGAGATAGG TTCCTGTGATTTTTCC TTCTTCTCTATAGAAT A	989	mir-198	GGTCCAGAGGGGAGATAGG	1209
mir_191	CGCCAACGGCTGGACA GCGGGCAACGGAATCC CAAAAGCAGCTGTTGT CTCCAGAGCATTCCAG CTGCGCTTGGATTTCG TCCCCTGCTCTCCTGC CTGAGC	990	mir-191	CAACGGAATCCCAAAAGCAGCT	1210
mir_191	CGCCAACGGCTGGACA GCGGGCAACGGAATCC CAAAAGCAGCTGTTGT CTCCAGAGCATTCCAG CTGCGCTTGGATTTCG TCCCCTGCTCTCCTGC CTGAGC	990	mir-191_Ruvkun	CAACGGAATCCCAAAAGCAGCTGT	1211
mir_206	GCTTCCCGAGGCCACA TGCTTCTTTATATCCC CATATGGATTACTTTG CTATGGAATGTAAGGA AGTGTGTGGTTTCGGC AAGT	991	mir-206	TGGAATGTAAGGAAGTGTGTGG	1212
mir-94/mir-106b	AGCCCTGCCGGGGCTA AAGTGCTGACAGTGCA GATAGTGGTCCTCTCC GTGCTACCGCACTGTG GGTACTTGCTGCTCCA GCAGGGCA	992	miR-94	AAAGTGCTGACAGTGCAGAT	1213
mir-94/mir-106b	AGCCCTGCCGGGGCTA AAGTGCTGACAGTGCA GATAGTGGTCCTCTCC GTGCTACCGCACTGTG GGTACTTGCTGCTCCA GCAGGGCA	992	miR-106b (RFAM-M. mu.)	TAAAGTGCTGACAGTGCAGAT	1214
mir_184	CCAGTCACGTCCCCTT ATCACTTTTCCAGCCC AGCTTTGTGACTGTAA GTGTTGGACGGAGAAC TGATAAGGGTAGGTGA TTGAC	993	miR-184	TGGACGGAGAACTGATAAGGGT	1215
mir_195	AGCTTCCCTGGCTCTA GCAGCACAGAAATATT GGCACAGGGAAGCGAG TCTGCCAATATTGGCT GTGCTGCTCCAGGCAG GGTGGTG	994	miR-195	TAGCAGCACAGAAATATTGGC	1216
mir_193	ATGGGAGCTGAGGGCT GGGTCTTTGCGGGCGA GATGAGGGTGTCGGAT CAACTGGCCTACAAAG TCCCAGTTCTCGGCCC CC	995	miR-193	AACTGGCCTACAAAGTCCCAG	1217

mir_185	AGGGGGCGAGGGATTG GAGAGAAAGGCAGTTC CTGATGGTCCCCTCCC CAGGGGCTGGCTTTCC TCTGGTCCTTCCCTCC CA	996	miR-185	TGGAGAGAAAGGCAGTTC	1218
mir_188	TCCCTGCTCCCTCTCT CACATCCCTTGCATGG TGGAGGGTGAGCTTTC TGAAAACCCCTCCCAC ATGCAGGGTTTGCAGG ATGGCGAGCCT	997	miR-188	CATCCCTTGCATGGTGGAGGGT	1219
mir_197	GGGGCTGTGCCGGGTA GAGAGGGCAGTGGGAG GTAAGAGCTCTTCACC CTTCACCACCTTCTCC ACCCAGCATGGCCG	998	miR-197a	TTCACCACCTTCTCCACCCAGC	1220
mir_194_1	CTTATATGTTTAATGG TGTTATCAAGTGTAAC AGCAACTCCATGTGGA CTGTGTACCAATTTCC AGTGGAGATGCTGTTA CTTTTGATGGTTACCA ACTTGCTACAATATAA A	999	miR-194	TGTAACAGCAACTCCATGTGGA	1221
mir_208	TTCCTGTGACGGGCGA GCTTTTGGCCCGGGTT ATACCTGATGCTCACG TATAAGACGAGCAAAA AGCTTGTTGGTCAGAG GAG	1000	miR-208	ATAAGACGAGCAAAAAGCTTGT	1222
mir_194_2	AATTGGTTCCCGCCCC CTGTAACAGCAACTCC ATGTGGAAGTGCCCAC TGGTTCCAGTGGGGCT GCTGTTATCTGGGGCG AGGGCCAGTAC	1001	miR-194	TGTAACAGCAACTCCATGTGGA	1221
mir_139	GGGACTGGCTCAGGTG TATTCTACAGTGCACG TGTCTCCAGTGTGGCT CGGAGGCTGGAGACGC GGCCCTGTTGGAGTAA CAACTGAAGCCGGAGT CT	1002	miR-139	TCTACAGTGCACGTGTCT	1223
mir-200b	GTGGCCATCTTACTGG GCAGCATTGGATGGAG TCAGGTCTCTAATACT GCCTGGTAATGATGAC GGCGGAG	1003	miR-200a (RFAM-Human)	CTCTAATACTGCCTGGTAATGATG	1224
mir-200b	GTGGCCATCTTACTGG GCAGCATTGGATGGAG TCAGGTCTCTAATACT GCCTGGTAATGATGAC GGCGGAG	1003	miR-200b (Michael et al)	TAATACTGCCTGGTAATGATGA	1225
mir-200b	GTGGCCATCTTACTGG GCAGCATTGGATGGAG TCAGGTCTCTAATACT GCCTGGTAATGATGAC GGCGGAG	1003	miR-200b	TAATACTGCCTGGTAATGATGAC	1226
mir-200a	CGGGCCCCTGTGAGCA TCTTACCGGACAGTGC	1004	miR-200a	TAACACTGTCTGGTAACGATG	1227

	TGGATTTCCCAGCTTG ACTCTAACACTGTCTG GTAACGATGTTCAAAG GTGACCCGC				
mir-200a	CGGGCCCCTGTGAGCA TCTTACCGGACAGTGC TGGATTTCCCAGCTTG ACTCTAACACTGTCTG GTAACGATGTTCAAAG GTGACCCGC	1004	miR-200a (RFAM-M. mu.)	TAACACTGTCTGGTAACGATGT	1228
mir-240* (Kosik)	TTGAGCGGGGGTCAAG AGCAATAACGAAAAAT GTTTGTCATAAACCGT TTTTCATTATTGCTCC TGACCTCCTCTCATTT G	1005	mir-240* (Kosik)	TCAAGAGCAATAACGAAAAATGT	1229
mir-232* (Kosik)	CAGAGCCTGGAGTGGG GGGGCAGGAGGGGCTC AGGGAGAAAGTGCATA CAGCCCCTGGCCCTCT CTGCCCTTCCGTCCCC TGCTCTT	1006	mir-232* (Kosik)	CTGGCCCTCTCTGCCCTTCCGT	1230
mir-227* (Kosik)/mi r-226* (Kosik)	TGACTATGCCTCCCCG CATCCCCTAGGGCATT GGTGTAAAGCTGGAGA CCCACTGCCCCAGGTG CTGCTGGGGGTTGTAG TCT	1007	mir-226* (Kosik)	ACTGCCCCAGGTGCTGCTGG	1231
mir-227* (Kosik)/mi r-226* (Kosik)	TGACTATGCCTCCCCG CATCCCCTAGGGCATT GGTGTAAAGCTGGAGA CCCACTGCCCCAGGTG CTGCTGGGGGTTGTAG TCT	1007	mir-324-3p_Ruvkun	CCACTGCCCCAGGTGCTGCTGG	1232
mir-227* (Kosik)/mi r-226* (Kosik)	TGACTATGCCTCCCCG CATCCCCTAGGGCATT GGTGTAAAGCTGGAGA CCCACTGCCCCAGGTG CTGCTGGGGGTTGTAG TCT	1007	mir-227* (Kosik)	CGCATCCCCTAGGGCATTGGTGT	1233
mir-244* (Kosik)	ACGGCTGTCCTCTCCA ACAATATCCTGGTGCT GAGTGATGACTCAGGC GACTCCAGCATCAGTG ATTTTGTTGAAGAGGG CAGCTGCCA	1008	mir-244* (Kosik)	TCCAGCATCAGTGATTTTGTTGA	1234
mir-224* (Kosik)	TGGTACTTGGAGAGAG GTGGTCCGTGGCGCGT TCGCTTTATTTATGGC GCACATTACACGGTCG ACCTCTTTGCAGTATC TA	1009	mir-224* (Kosik)	GCACATTACACGGTCGACCTCT	1235
mir-248* (Kosik)	GAAACTGGGCTCAAGG TGAGGGGTGCTATCTG TGATTGAGGGACATGG TTAATGGAATTGTCTC ACACAGAAATCGCACC CGTCACCTTGGCCTAC TTATCA	1010	mir-248* (Kosik)	TCTCACACAGAAATCGCACCCGTC	1236
ribosomal protein	ATCTATGAAGGCCAAG TGGAGGTGACTGGTGA	1011	hypothetical	AGCCAGGTGCCTTCACCTGCT	1155

L5/ hypothetical miRNA 168_2	TGAATACAATGTGGAA AGCATTGATGGTCAGC CAGGTGCCTTCACCTG CTATTTGGATG		miRNA-168		
hypothetical miRNA-177_3	CAGTGCTTTCAGCAAG AGGGTGGTACCACATG TCTTCAAAATGAAACG TCTCTTGGAGACAAAC ATGCTACTCTCACTGC AGTTGAAAAAAGTCAC A	1012	hypothetical miRNA-177	AGACAAACATGCTACTCTCAC	1164
mir-138_3	ATGGTGTGGTGGGGCA GCTGGTGTTGTGAATC AGGCCGTTGCCAATCA GAGAACGGCTACTTCA CAACACCAGGGCCACA CCACACTACA	1013	mir-138	AGCTGGTGTTGTGAATC	256
mir-138_3	ATGGTGTGGTGGGGCA GCTGGTGTTGTGAATC AGGCCGTTGCCAATCA GAGAACGGCTACTTCA CAACACCAGGGCCACA CCACACTACA	1013	mir-138_Ruvkun	AGCTGGTGTTGTGAATCAGGCCG	1127
mir-138_4	GGAATCAGATTAGCTG GTGTTGTGAATCTCCA TCTGAACTAAGATGGA CAGTAAGAGAATCCCA ATATCTTAGTAAATCT GTTACT	1014	mir-138	AGCTGGTGTTGTGAATC	256
mir-181b_2	ATGGCTGCACTCAACA TTCATTGCTGTCGGTG GGTTTGAGTCTGAATC AACTCACTGATCAATG AATGCAAACTGCGGAC CAAA	1015	mir-181b	AACATTCATTGCTGTCGGTGGGTT	260
mir-219_1	GGGCCGCGGCTCCTGA TTGTCCAAACGCAATT CTCGAGTCTATGGCTC CGGCCGAGAGTTGAGT CTGGACGTCCCGAGCC GCCGCCCCC	1016	mir-219	TGATTGTCCAAACGCAATTCT	271
mir-105_2	TGTGTGTGCATCGTGG TCAAATGCTCAGACTC CTGTGGTGGCTGCTTA TGCACCACGGATGTTT GAGCATGTGCTATGGT GTCTACTT	1017	miR-105 (Mourelatos)	TCAAATGCTCAGACTCCTGT	1185
hypothetical miRNA 120_2	TCTTCCAGTCATCCTG AGGTAGATATCATACA GGAATGCTGGGGCCTT ATGGCTTACAGCAATC CAGTAATGATATAAAA GATGATTGGAGGTTA	1018	hypothetical miRNA-120	TTACAGCAATCCAGTAATGAT	1138
cezanne 2/ hypothetical miRNA-180	ACGCAGTCCTGTCAGA CTTTGTTCGGTCCACG GGGGCAGAACCTGGTC TGGCCAGAGACCTGCT GG	1019	hypothetical miRNA-180	TCCTGTCAGACTTTGTTCGGT	1237
mir-103_2	TTGTGCTTTCAGCTTC TTTACAGTGCTGCCTT GTAGCATTCAGGTCAA	1020	mir-103	AGCAGCATTGTACAGGGCTATGA	225

	GCAGCATTGTACAGGG CTATGAAAGAACCA				
mir-147 (Sanger)	AATCTAAAGACAACAT TTCTGCACACACACCA GACTATGGAAGCCAGT GTGTGGAAATGCTTCT GCTAGATT	1021	miR-147 (RFAM-Human)	GTGTGTGGAAATGCTTCTGC	1238
mir-224 (Sanger)	GGGCTTTCAAGTCACT AGTGGTTCCGTTTAGT AGATGATTGTGCATTG TTTCAAAATGGTGCCC TAGTGACTACAAAGCC C	1022	miR-224 (RFAM-Human)	CAAGTCACTAGTGGTTCCGTTTA	1239
mir-134 (Sanger)	CAGGGTGTGTGACTGG TTGACCAGAGGGGCAT GCACTGTGTTCACCCT GTGGGCCACCTAGTCA CCAACCCTC	1023	miR-134 (RFAM-Human)	TGTGACTGGTTGACCAGAGGG	1240
mir-146 (Sanger)	CCGATGTGTATCCTCA GCTTTGAGAACTGAAT TCCATGGGTTGTGTCA GTGTCAGACCTCTGAA ATTCAGTTCTTCAGCT GGGATATCTCTGTCAT CGT	1024	miR-146 (RFAM-Human)	TGAGAACTGAATTCCATGGGTT	1241
mir-150 (Sanger)	CTCCCCATGGCCCTGT CTCCCAACCCTTGTAC CAGTGCTGGGCTCAGA CCCTGGTACAGGCCTG GGGGACAGGGACCTGG GGAC	1025	miR-150 (RFAM-Human)	TCTCCCAACCCTTGTACCAGTG	1242
mir-30e (RFAM/mmu)	TGGGCAGTCTTTGCTA CTGTAAACATCCTTGA CTGGAAGCTGTAAGGT GTTCAGAGGAGCTTTC AGTCGGATGTTTACAG CGGCAGGCTGCCACGG	1026	miR-30e (RFAM-M. mu.)	TGTAAACATCCTTGACTGGA	1243
mir-30e (RFAM/mmu)	TGGGCAGTCTTTGCTA CTGTAAACATCCTTGA CTGGAAGCTGTAAGGT GTTCAGAGGAGCTTTC AGTCGGATGTTTACAG CGGCAGGCTGCCACGG	1026	miR-97 (Michael et al)	TGTAAACATCCTTGACTGGAAG	1244
mir-296 (RFAM/mmu)	GACCCTTCCAGAGGGC CCCCCCTCAATCCTGT TGTGCCTAATTCAGAG GGTTGGGTGGAGGCTC TCCTGAAGGGC	1027	miR-296 (RFAM-M. mu.)	AGGGCCCCCCCTCAATCCTGT	1245
mir-299 (RFAM/mmu)	CGGTACTTGAAGAAAT GGTTTACCGTCCCACA TACATTTTGAATATGT ATGTGGGATGGTAAAC CGCTTCTTGGTATCC	1028	miR-299 (RFAM-M. mu.)	TGGTTTACCGTCCCACATACAT	1246
mir-301 (RFAM/mmu)	TTACTGCTAACGAATG CTCTGACTTTATTGCA CTACTGTACTTTACAG CTAGCAGTGCAATAGT ATTGTCAAAGCATCTG AAAGCAGGA	1029	miR-301 (RFAM-M. mu.)	CAGTGCAATAGTATTGTCAAAGC	1247
mir-301 (RFAM/mmu)	TTACTGCTAACGAATG CTCTGACTTTATTGCA	1029	mir-301 Ruvk	CAGTGCAATAGTATTGTCAAAGCA T	1248

	CTACTGTACTTTACAG CTAGCAGTGCAATAGT ATTGTCAAAGCATCTG AAAGCAGGA		un		
mir-302 (RFAM/mmu)	CAAGACTGGGCTCCCC ACCACTTAAACGTGGA TGTACTTGCTTTGAAA CTAAAGAAGTAAGTGC TTCCATGTTTTGGTGA TGGTAAGTCTTCCTTT	1030	miR-302 (RFAM-M. mu.)	TAAGTGCTTCCATGTTTTGGTGA	1249
mir-34a (RFAM/mmu)	TGAGTCTAGTTACTAG GCAGTGTAGTTAGCTG ATTGCTAATAGTACCA ATCACTAACCACACGG CCAGGTAAAAAGATTT G	1031	mir-34c (RFAM)	AGGCAGTGTAGTTAGCTGATTG	1250
mir-34a (RFAM/mmu)	TGAGTCTAGTTACTAG GCAGTGTAGTTAGCTG ATTGCTAATAGTACCA ATCACTAACCACACGG CCAGGTAAAAAGATTT G	1031	miR-34a (RFAM-M. mu.)	AGGCAGTGTAGTTAGCTGATTGC	1251
mir_320	CGGCGCTTCGCTCCCC TCCGCCTTCTCTTCCC GGTTCTTCCCGGAGTC GGGAAAAGCTGGGTTG AGAGGGCGAAAAAGGA TGAGGTGACTG	1032	miR-320	AAAAGCTGGGTTGAGAGGGCGAA	1252
mir-321_1	ATGGATAAGGCATTGG CCTCCTAAGCCAGGGA TTGTGGGTTCGAGTCC CATCTGGGGTGGCCTG TGACTTTTGTCCTTTT T	1033	miR-321-1	TAAGCCAGGGATTGTGGGTTC	1253
mir-135b (Ruvkun)	TGCTGTGGCCTATGGC TTTTCATTCCTATGTG ATTGCTGTCCCAAACT CATGTAGGGCTAAAAG CCATGGGCTACAGTG	1034	mir-135b (Ruvkun)	TATGGCTTTTCATTCCTATGTG	1254
mir-151* (Ruvkun)	TTTCCTGCCCTCGAGG AGCTCACAGTCTAGTA TGTCTCATCCCCTACT AGACTGAAGCTCCTTG AGGACAGGGAT	1035	mir-151 (human)	ACTAGACTGAAGCTCCTTGAGG	1255
mir-151* (Ruvkun)	TTTCCTGCCCTCGAGG AGCTCACAGTCTAGTA TGTCTCATCCCCTACT AGACTGAAGCTCCTTG AGGACAGGGAT	1035	mir-151* (Ruvkun)	TCGAGGAGCTCACAGTCTAGTA	1256
mir-340 (Ruvkun)	TGTACCTGGTGTGATT ATAAAGCAATGAGACT GATTGTCATATGTCGT TTGTGGGATCCGTCTC AGTTACTTTATAGCCA TACCTGGTATC	1036	mir-340 (Ruvkun)	TCCGTCTCAGTTACTTTATAGCC	1257
mir-331 (Ruvkun)	TGTTTGGGTTTGTTCT AGGTATGGTCCCAGGG ATCCCAGATCAAACCA GGCCCCTGGGCCTATC CTAGAACCAACCTAAG CT	1037	mir-331 (Ruvkun)	GCCCCTGGGCCTATCCTAGAA	1258

mir_200c (RFAM)	GCGGGGGCCCTCGTCT TACCCAGCAGTGTTTG GGTGCGGTTGGGAGTC TCTAATACTGCCGGGT AATGATGGAGGCCCCT GT	1038	mir-200c (RFAM)	AATACTGCCGGGTAATGATGGA	1259
mir_34b (RFAM)	TGCTCGGTTTGTAGGC AGTGTCATTAGCTGAT TGTACTGTGGTGGTTA CAATCACTAACTCCAC TGCCATCAAAACAAGG CACAGCATCAC	1039	mir-34b (RFAM)	AGGCAGTGTCATTAGCTGATTG	1260
mir_339_1 (RFAM)	AGGGGCGGGCGGCCGC TCTCCCTGTCCTCCAG GAGCTCACGTGTGCCT GCCTGTGAGCGCCTCG ACGACAGAGCCGCGCC TGCCCCA	1040	mir-339 (RFAM)	TCCCTGTCCTCCAGGAGCTCA	1261
mir_339_2 (RFAM)	AGGGGCGGGGCGGCCG CTCTCCCTGTCCTCCA GGAGCTCACGTGTGCC TGCCTGTGAGCGCCTC GACGACAGAGCCGGCG CCTGCCCCA	1041	mir-339 (RFAM)	TCCCTGTCCTCCAGGAGCTCA	1261
mir-325 (Ruvkun)	AGTGCTTGGTTCCTAG TAGGTGTCCAGTAAGT GTTTGTGACATAATTT GTTTATTGAGGACCTC CTATCAATCAAGCACT GTGCTAGGCTCTGG	1042	mir-325 (human)	CCTAGTAGGTGTCCAGTAAGTGT	1262
mir-326 (Ruvkun)	CTCATCTGTCTGTTGG GCTGGAGGCAGGGCCT TTGTGAAGGCGGGTGG TGCTCAGATCGCCTCT GGGCCCTTCCTCCAGC CCCGAGGCGGATT	1043	miR-326 (Ruvkun)	CCTCTGGGCCCTTCCTCCAG	1263
mir-326 (Ruvkun)	CTCATCTGTCTGTTGG GCTGGAGGCAGGGCCT TTGTGAAGGCGGGTGG TGCTCAGATCGCCTCT GGGCCCTTCCTCCAGC CCCGAGGCGGATT	1044	mir-326 (human)	CCTCTGGGCCCTTCCTCCAGC	1264
mir-329-1 (Ruvkun)	TGGTACCTGAAGAGAG GTTTTCTGGGTTTCTG TTTCTTTAATGAGGAC GAAACACACCTGGTTA ACCTCTTTTCCAGTAT CAAATCC	1045	mir-329 (human)	AACACACCTGGTTAACCTCTTT	1265
mir-329-2 (Ruvkun)	TGGTACCTGAAGAGAG GTTTTCTGGGTTTCTG TTTCTTTATTGAGGAC GAAACACACCTGGTTA ACCTCTTTTCCAGTAT CAAATCC	1046	mir-329 (human)	AACACACCTGGTTAACCTCTTT	1265
mir-330 (Ruvkun)	CTTTGGCGATCACTGC CTCTCTGGGCCTGTGT CTTAGGCTCTGCAAGA TCAACCGAGCAAAGCA CACGGCCTGCAGAGAG GCAGCGCTCTGC	1047	mir-330 (human)	GCAAAGCACACGGCCTGCAGAGA	1266

mir-337 (Ruvkun)	GTAGTCAGTAGTTGGG GGGTGGGAACGGCTTC ATACAGGAGTTGATGC ACAGTTATCCAGCTCC TATATGATGCCTTTCT TCATCCCCTTCAA	1048	mir-337 (human)	TCCAGCTCCTATATGATGCCTTT	1267
mir-345 (Ruvkun)	CTGCTGACTCCTAGTC CAGGGCTCGTGATGGC TGGTGGGCCCTGAACG AGGGGTCTGGAGGCCT GGGTTTGA	1049	mir-345 (human)	TGCTGACTCCTAGTCCAGGGC	1268
mir-346 (Ruvkun)	TCTGTGTTGGGCGTCT GTCTGCCCGCATGCCT GCCTCTCTGTTGCTCT GAAGGAGGCAGGGGCT GGGCCTGCAGCTGCCT GGGCAGAGCGGCTCCT	1050	mir-346 (human)	TGTCTGCCCGCATGCCTGCCTCT	1269
mir-187	GGTCGGGCTCACCATG ACACAGTGTGAGACCT CGGGCTACAACACAGG ACCCGGGCGCTGCTCT GACCCCTCGTGTCTTG TGTTGCAGCCGGAGGG ACGCAGGTCCGCA	1051	miR-187 (RFAM-Human)	TCGTGTCTTGTGTTGCAGCCG	1270
mir-187	GGTCGGGCTCACCATG ACACAGTGTGAGACCT CGGGCTACAACACAGG ACCCGGGCGCTGCTCT GACCCCTCGTGTCTTG TGTTGCAGCCGGAGGG ACGCAGGTCCGCA	1051	mir-187	TCGTGTCTTGTGTTGCAGCCGG	276
miR-24-1	CCCTCCGGTGCCTACT GAGCTGATATCAGTTC TCATTTTACACACTGG CTCAGTTCAGCAGGAA CAG	1052	miR-189 (RFAM-Human)	GTGCCTACTGAGCTGATATCAGT	1271
miR-24-1	CCCTCCGGTGCCTACT GAGCTGATATCAGTTC TCATTTTACACACTGG CTCAGTTCAGCAGGAA CAG	1052	mir-24	TGGCTCAGTTCAGCAGGAACAG	264
mir-215	TGGTATACAGGAAAAT GACCTATGAATTGACA GACAATATAGCTGAGT TTGTCTGTCATTTCTT TAGGCCAATATTCTGT ATGACTGTGCTACTT	1053	mir-215	ATGACCTATGAATTGACAGAC	278

A list of mouse pri-miRNAs and the mature miRNAs predicted to derive from them is shown in Table 61. "Pri-miRNA name" indicates the gene name for each of the pri-miRNAs, and "pri-miRNA sequence" indicates the sequence of the predicted primary miRNA transcript. Also given in table 61 are the name and sequence of the mature miRNA derived from the pri-miRNA. Mature miRNA sequences from pri-miRNA precursors have been proposed by several groups; consequently, for a given pri-miRNA sequence, several miRNAs may be disclosed and given unique names, and thus a given pri-miRNA sequence may occur repeatedly in the table.